

# JOURNAL OF ANATOMY

ORIGINALLY THE JOURNAL OF  
ANATOMY AND PHYSIOLOGY

QL  
801  
J7

CONDUCTED ON BEHALF OF THE ANATOMICAL SOCIETY  
OF GREAT BRITAIN AND IRELAND BY

D. V. DAVIES

K. C. RICHARDSON

F. GOLDBY

E. W. WALLS

W. J. HAMILTON

A. G. M. WEDDELL

G. A. G. MITCHELL

J. D. BOYD (EDITOR)

VOLUME 89

JANUARY 1955—OCTOBER 1955

CAMBRIDGE  
AT THE UNIVERSITY PRESS  
1955

*Printed in Great Britain at the University Press, Cambridge  
(Brooke Crutchley, University Printer)  
and published by the Cambridge University Press  
London: Bentley House, N.W.1  
American Branch: New York  
Agents for Canada, India, and Pakistan: Macmillan*



# CONTENTS

## PART 1—JANUARY 1955

	PAGE
Selective and histochemical staining of the otolithic membranes, cupulae and tectorial membrane of the inner ear. By GEORGE B. WISLOCKI and AARON J. LADMAN . . . . .	3
Incorporation of <sup>35</sup> S-DL-methionine in mouse tissues as indicated by autoradiographs. I. Testis, epididymis and seminal vesicle. By A. GLUCKSMANN, ALMA HOWARD and S. R. PELC . . . . .	13
A quantitative study of the mamillary bodies and their connexions. By R. W. GUILLERY . . . . .	19
The organization of the visual cortex in the cat. By D. A. SHOLL . . . . .	33
Further observations on the behaviour of nuclear structures during depletion and restoration of Nissl material. By HUGH A. LINDSAY and MURRAY L. BARR . . . . .	47
Enumeration of glomeruli in the kidney of the dog. By R. V. SELLWOOD and E. B. VERNEY . . . . .	63
The influence of cortisone on the structure and growth of bone. By H. A. SISSONS and G. J. HADFIELD . . . . .	69
The effects of partial or complete excision of the epiphyseal cartilage of the rabbit. By P. A. RING . . . . .	79
Some observations on the intracellular lipid in the kidney of the cat. By MARY C. LOBBAN . . . . .	92
Arterio-venous anastomoses in the skin of the head and ears of the calf. By A. MYFANWY GOODALL . . . . .	100
The anatomy and functional significance of the vascularization of the adrenal gland in the rhesus monkey ( <i>Macaca mulatta</i> ). By R. G. HARRISON and C. W. ASLING . . . . .	106
Contracture and intussusceptive growth in the healing of extensive wounds in mammalian skin. By R. E. BILLINGHAM and P. B. MEDAWAR . . . . .	114
Mitotic activity in the vaginal epithelium of the mouse following local oestrogenic stimulation. By J. D. BIGGERS and P. J. CLARINGBOLD . . . . .	124
REVIEW . . . . .	132
BOOKS RECEIVED . . . . .	132

## PART 2—APRIL 1955

	PAGE
The placodal relations of the neural crest in the domestic cat. By GWEN HALLEY	133
Cell degeneration during normal ontogenesis of the rabbit brain. By BENGT KÄLLÉN . . . . .	153
Studies on the innervation of skin. I. The origin, course, and number of sensory nerves supplying the rabbit ear. By G. WEDDELL, W. PALLIE and ELIZABETH PALMER . . . . .	162
Studies on the innervation of skin. II. The number, size and distribution of hairs, hair follicles and orifices from which the hairs emerge in the rabbit ear. By G. WEDDELL and W. PALLIE . . . . .	175
A note on the innervation of human dentine. By RALPH COCKER and JEAN M. HATTON . . . . .	189
The perivascular spaces of the mammalian central nervous system and their relation to the perineuronal and subarachnoid spaces. By D. H. M. WOOLLAM and J. W. MILLEN . . . . .	193
Observations on the development of microglia together with a note on the influence of cortisone. By E. J. FIELD . . . . .	201
The arterial supply of the human prostate and seminal vesicles. By E. J. CLEGG .	209
Observations on the valves of Houston in the human embryo and foetus. By P. H. S. SILVER . . . . .	217
Some factors influencing angulation of the neck of the mammalian talus. By C. H. BARNETT . . . . .	225
Excision and reimplantation of the epiphyseal cartilage of the rabbit. By P. A. RING . . . . .	231
Alkaline phosphatase activity in the developing teeth of the rat. By N. B. B. SYMONS . . . . .	238
The arrangement of the ansa spiralis of the sheep colon. By R. N. SMITH . .	246
The part played by the tongue in mastication and deglutition. By SHAFIK ABD-EL-MALEK . . . . .	250
IN MEMORIAM: FREDERIC WOOD JONES . . . . .	255
REVIEWS . . . . .	268

## PART 3—JULY 1955

The behaviour of autografts and homografts of vaginal tissue in rabbits. By P. L. KROHN . . . . .	269
Cellular changes in lymph nodes and spleen following skin homografting in the rabbit. By R. J. SCOTHORNE and I. A. MCGREGOR . . . . .	283
Adreno-cortical histogenesis in the rat: with observations on lipid and ascorbic acid distribution. By J. D. LEVER . . . . .	293



	PAGE
Hypothalamic neurosecretion in the dog and cat, with particular reference to the identification of neurosecretory material with posterior lobe hormone. By J. C. SLOPER . . . . .	301
Studies on the innervation of skin. III. The patterned arrangement of the spinal sensory nerves to the rabbit ear. By G. WEDDELL, D. A. TAYLOR and C. M. WILLIAMS . . . . .	317
The surface features of the brain of the humpback whale ( <i>Megaptera novaeangliae</i> ) By A. S. BREATHNACH . . . . .	343
The position of the hallux in West Africans. By N. A. BARNICOT and R. H. HARDY . . . . .	355
The form and function of the carpo-metacarpal joint of the thumb. By J. R. NAPIER . . . . .	362
The thoraco-lumbar mortice joint. By P. R. DAVIS . . . . .	370
The role of the scalene and sternomastoid muscles in breathing in normal subjects. An electromyographic study. By E. J. M. CAMPBELL . . . . .	378
The broncho-pulmonary segments in the sheep. By W. C. D. HARE . . . . .	387
IN MEMORIAM:	
SIR ARTHUR KEITH . . . . .	403
JAMES WHILLIS . . . . .	419
REVIEWS . . . . .	422
BOOKS RECEIVED . . . . .	424

## PART 4—OCTOBER 1955

Esterase activity in the skin of mammals. By WILLIAM MONTAGNA and VICTOR R. FORMISANO . . . . .	425
A histochemical study of the seminal vesicle of the sheep. By R. N. C. AITKEN . . . . .	430
Histo- and biochemical studies of alkaline phosphatase in guinea-pigs with experimentally produced obstructive jaundice. By F. JACOBY and B. F. MARTIN . . . . .	440
The behaviour of implantation grafts of bladder mucosa. By F. R. JOHNSON and R. M. H. McMINN . . . . .	450
Ossification and growth of the distal ulnar epiphysis of the rabbit. By P. A. RING . . . . .	457
The renal ducts of Bellini. By F. DURAN-JORDA . . . . .	464
Quantitative analysis of cell types in mammalian neo-cortex. By N. L. MITRA . . . . .	467
The postnatal development of the human cardiac ventricles. By E. N. KEEN . . . . .	484
The carotid labyrinth in <i>Hyla aurea</i> , with a note on that in <i>Leiopelma hochstetteri</i> . By J. B. CARMAN . . . . .	503

	PAGE
The morphology of the sternomastoid and trapezius muscles. By J. MCKENZIE	526
Observations on endocranial casts of recent and fossil cetaceans. By A. S. BREATHNACH . . . . .	532
The blood capillary system of the odontoblast layer of the dental pulp. By W. WARWICK JAMES . . . . .	547
The insertion of the biceps femoris. By R. S. SNEATH . . . . .	550
REVIEWS . . . . .	554
PROCEEDINGS . . . . .	557
INDEX . . . . .	582
SUPPLEMENTARY INDEX OF PROCEEDINGS . . . . .	586

THE  
FREDERICK WOOD JONES VOLUME  
OF  
THE JOURNAL OF ANATOMY

---

VOLUME 89 OF THE *JOURNAL OF ANATOMY*

IS DEDICATED BY

THE COUNCIL OF THE ANATOMICAL SOCIETY OF  
GREAT BRITAIN AND IRELAND

TO THE MEMORY OF

PROFESSOR F. WOOD JONES  
MEMBER OF THE SOCIETY FROM 1902 TO 1954





# SELECTIVE AND HISTOCHEMICAL STAINING OF THE OTOLITHIC MEMBRANES, CUPULAE AND TECTORIAL MEMBRANE OF THE INNER EAR

BY GEORGE B. WISLOCKI AND AARON J. LADMAN\*

*Department of Anatomy, Harvard Medical School, Boston, Massachusetts*

The present investigation reveals that the tectorial membrane, the gelatinous substance of the otolithic membranes and the cupulae of the internal ear are stained intensely and selectively by the alum-haematoxylin element of Gomori's chrome alum-haematoxylin and phloxine stain and by his aldehyde fuchsin stain. They react strongly also with the periodic acid-Schiff technique following exposure to saliva. These reactions are similar to those shown by several other structures of the central nervous system, namely, the hypophysial Herring substance (Bargmann, 1949; Bargmann & Scharrer, 1951), the subcommissural organ and Reissner's fibre (Wislocki & Leduc, 1952*a, b*; Bargmann & Schiebler, 1952) and the ocular ciliary zonula (Wislocki, 1952). Brief mention of the selective staining of the tectorial membrane (Wislocki, 1952) and of the otolithic membranes and cupulae has appeared elsewhere (Wislocki & Ladman, 1954). The present paper gives a more complete account of the staining of these otic structures by a number of histochemical methods and selective stains and discusses the possible significance of the results.

## MATERIAL AND METHODS

The material consisted of two foetal mouse heads of 17 days of gestation, six heads of newborn mice and the temporal bones of two 3½-month-old mice. It was supplemented by two temporal bones obtained from a fresh human foetus of 17 cm. crown-rump length.

The material was fixed according to the prescriptions of the different staining procedures. The temporal bones of the 3½-month-old mice were fixed in a saturated solution of mercuric chloride in 10 % formalin and decalcified in 5 % trichloroacetic acid before sectioning them. The less densely calcified heads of the foetal specimens and newborn mice proved soft enough to section them without resort to a special decalcifying agent.

The heads were embedded in paraffin and sectioned at 5  $\mu$ , either in the horizontal or the sagittal plane. The former proved more favourable for encompassing all the structures in question within a single section.

Gomori's chrome alum-haematoxylin and phloxine method (1941), and his aldehyde fuchsin stain counterstained with orange G and light green as modified by Halmi (1952), were applied to deparaffinized sections of material fixed in mercuric chloride and formalin, Rossman's fluid (a saturated solution of picric acid in absolute alcohol: 90 ml.; formaldehyde: 10 ml.), Bouin's fluid, Orth's fixative and Zenker's acetic acid fixative.

\* Research Fellow of the American Cancer Society, Inc., upon recommendation of the Committee on Growth of the National Research Council, 1952-4.



The periodic acid-Schiff method of McManus (1946) and Hotchkiss (1948) was applied to deparaffinized sections of the same blocks. Prior to staining them, the sections were placed in saliva for 2 or 3 hr. to remove glycogen. Other saliva-treated sections were stained with Schiff's reagent without previous oxidation with periodic acid.

Pap's ammoniacal silver nitrate method for the demonstration of fibrous reticulum, as modified by Mitchell & Wislocki (1944), and Weigert's resorcin-fuchsin stain for elastic tissue were applied to sections from the same blocks.

Other deparaffinized sections of internal ears fixed in 4% basic lead acetate or Zenker's acetic acid fixative were stained in either a 1% aqueous solution of methylene blue or toluidin blue for the demonstration respectively of possible cytoplasmic basophilia and metachromasia. A series of sections of one of the labyrinths of the human foetus listed above was stained in solutions of methylene blue of graded pH as described by Dempsey, Bunting, Singer & Wislocki (1947).

Sections of two heads of newborn mice fixed in a solution of 1% trichloroacetic acid in 80% ethanol and in Zenker's acetic acid fluid were stained by the methods of Barrnett & Seligman (1952, 1954) for protein-bound sulph-hydryl and disulphide groups.

#### OBSERVATIONS

*Staining with chrome alum-haematoxylin and aldehyde fuchsin.* Similar staining results were obtained by both methods at the three stages of development of the mouse and in the human tectorial membrane. Consequently, it is unnecessary to represent each structure at every stage by both methods.

Pl. 1, fig. 1, illustrates a typical horizontal section through the internal ear of a newborn mouse, revealing a crista ampullaris, the utricle and saccule with their maculae, and a portion of the cochlea with the developing organ of Corti. The section was stained by the chrome alum-haematoxylin and phloxine method. The three regions of the section contained in rectangles coincide approximately with the areas of figs. 2 and 19, 3 and 14, and 6 and 18 in Pls. 1-3. Reference to Pl. 3, figs. 14, 18 and 19, illustrates that the otolithic membrane of the macula of the utricle, the developing tectorial membrane and the cupula of a crista ampullaris are stained selectively blue by the alum-haematoxylin component of Gomori's stain. Pl. 1, fig. 4, and Pl. 3, fig. 15, of the macula sacculi of a  $3\frac{1}{2}$ -month-old mouse reveal a similar selective staining of the otolithic membrane by the aldehyde fuchsin stain. These representative illustrations will serve to show that the tectorial membrane, cupulae and otolithic membranes are selectively stained.

Precisely what elements of these membranes are stained is not so easy to judge. The tectorial membrane is generally accepted as being composed of a fibrillar matrix and a jelly-like ground substance. The otolithic membrane is believed to consist of a layer of a gelatinous substance in the outer part of which there are numerous small bodies, the otoliths or otoconia, which are composed of a mixture of calcium carbonate and a protein. The cupulae are described as consisting of a jelly-like mass of substance containing pores or canals into which the hairs of the sensory cells of the cristae project. The cupulae are also described under some conditions as possessing a finely striated texture, an appearance which, according to Kolmer (1927), may be

a fixation artifact of the colloids of which they are composed. From examination of Pl. 3, figs. 14, 15, 18 and 19, it is apparent that no critical distinctions between the several components of these membranes can be made upon the basis of their staining by means of chrome alum-haematoxylin or aldehyde fuchsin. In the case of the otolithic membranes (Pl. 3, figs. 14, 15) it could be the protein of the otoliths that is stained, but it is equally possible that the gelatinous matrix precipitated upon the otoliths could account for the staining. Fibrillar shreds of selectively stained material appear to extend from the otolithic membrane on to the flagellae and the outer surface of the cells of the macula. The same relation of stained material to cells is faintly seen in the case of the cupula and crista (Pl. 3, fig. 19). In the developing organ of Corti, quite heavily stained, blue fibrils appear to connect the under surface of the tectorial membrane with the adjacent surface of Corti's organ, and thence bluish strands seem to penetrate between the columnar cells (Pl. 3, fig. 18). On the other hand, the bluish fibrils visible quite generally in the subepithelial connective tissue of the labyrinth (Pl. 3, figs. 14, 18 and 19) are apparently collagenous fibrils which are known to stain variably strongly with chrome alum-haematoxylin.

*The periodic acid-Schiff reaction.* By this procedure the tectorial membrane (Pl. 2, fig. 7), the otolithic membranes (Pl. 3, fig. 16) and the cupulae (Pl. 1, fig. 5; Pl. 3, fig. 17) are intensely stained. Since this staining occurs in sections which were exposed to saliva, it cannot be attributed to the presence of glycogen. Here, as with the previous stains, a decision cannot be reached in regard to which components of the membranes—whether the gelatinous substance or the fibrillar component, or both—are specifically stained. It is also possible that the protein of the otoliths may be stained. A basement membrane upon which the epithelium of the maculae and cristae rests also stains intensely (Pl. 3, figs. 16, 17). Similar staining is observed in the foetal and newborn mouse heads, as well as in the human tectorial membrane.

Sections stained with Schiff's reagent without prior oxidation with periodic acid reveal mild staining of the tectorial membrane, but none of the otolithic membranes.

*Metachromasia and basophilia.* For the investigation of these manifestations of staining, sections were used from ears fixed in 4% basic lead acetate, which is recommended for the preservation of acid mucopolysaccharides (Holmgren & Wilander, 1937; Wislocki, Bunting & Dempsey, 1947), or fixed in Zenker's acetic acid fluid. In sections of ears of newborn mice so fixed and then stained with toluidin blue, no metachromasia was observed in the tectorial and otolithic membranes or in the cupulae. In the ear of a 3½-month-old mouse which was decalcified in 5% trichloroacetic acid there was similarly no metachromasia of the structures in question, except in one section in which the staining was allowed to run overnight, when a somewhat reddish blue coloration developed. In all of the sections referred to, cartilage matrix and the granules of mast cells showed intense metachromasia, attributable to their acid mucopolysaccharide content, thus serving as test-objects for comparison with the membranes under investigation.

Basophilic staining, carried out on a series of sections stained in solutions of methylene blue of ascending pH (Dempsey *et al.* 1947), provided a further means of characterizing acid substances and of investigating the properties of these mem-



branes. Only a foetal human organ of Corti was stained in this manner. It shows moderate nuclear and some cytoplasmic basophilia at pH 4.3, but exhibits no basophilia of the tectorial membrane (Pl. 2, fig. 12). This indicates that the substance of the tectorial membrane is considerably less acid than the nucleoproteins which are moderately stained at this pH. Furthermore, the tectorial membrane does not appear to consist of a sulphated mucopolysaccharide, because these compounds stain intensely with basic dyes at pH 4 (Dempsey *et al.* 1947).

*Protein-bound sulph-hydryl and disulphide groups.* As a further means of attempting to characterize the substance of the membranes in question, sections were stained by the methods of Barrnett and Seligman for sulph-hydryl and disulphide groups. Upon staining for sulph-hydryls the membranes react only slightly. By the procedure for the demonstration of disulphide groups the response is greater. In the maculae and cristae the reaction tinges the gelatinous material of the otolithic membranes and cupulae, and it also colours the respective epithelia, particularly the distal margins of the cells and the hair bundles. Pl. 2, fig. 13, shows this for the macula and otolithic membrane. The tectorial membrane stains more deeply for disulphide groups (Pl. 2, fig. 8) than the substance of either the otolithic membranes or cupulae. Of particular interest is the relatively intense staining of the hair cells of Corti's organ in the newborn mouse (Pl. 2, figs. 8 (arrow), 10 and 11). The supranuclear cytoplasm and the developing hairs of these cells react intensely for disulphide groups (Pl. 2, figs. 10, 11).

The strength of the reaction for disulphide groups varies somewhat depending upon the fixative used; the deepest staining was obtained in internal ears fixed in Zenker's acetic acid fluid.

*Weigert's resorcin-fuchsin stain for elastic tissue.* By this method the otolithic membrane and cupulae are negative, but the tectorial membrane is moderately stained. However, in the photograph illustrating this staining (Pl. 2, fig. 9), the tectorial membrane appears darker than in the actual histological section. Moreover, under the microscope it is perceived that the membrane stains reddish rather than the usual bluish black colour characteristic of typical elastic tissue.

*Argyrophil reticular fibres.* Stained with ammoniacal silver nitrate for argyrophil fibrous reticulum, all of these otic membranes assume pale brownish or tan colours depending upon the fixatives used. In contrast to this, true collagenous reticular fibres, present in the stroma surrounding the labyrinth, are typically argyrophilic, assuming, variously, a deep brown or black appearance.

#### DISCUSSION

From the preceding observations it is apparent that the tectorial and otolithic membranes and the cupulae of the internal ear have very similar histological and histochemical properties. They stain similarly and selectively with the alum-haematoxylin component of Gomori's chrome alum-haematoxylin and phloxine stain and with Gomori's aldehyde fuchsin stain. In addition, they react intensely with the periodic acid-Schiff procedure. They also react mildly to moderately for protein-bound disulphide groups, but do not exhibit metachromasia with toluidin blue or stain at low pH with methylene blue. They stain poorly with the ammoniacal silver nitrate method for collagenous reticulum and negatively with Weigert's resorcin-



fuchsin stain for elastic tissue, excepting the tectorial membrane which stains moderately and atypically by the latter method.

The chemical nature of the selective staining with chrome alum-haematoxylin and aldehyde fuchsin is poorly understood. The intense staining with the periodic acid-Schiff technique, combined with saliva controls, precludes glycogen and indicates the presence in these structures of a mucopolysaccharide or glycoprotein (cf. Leblond, 1950). The mild reaction of the tectorial membrane with the Schiff reagent alone, without oxidation with periodic acid, indicates the presence in this carbohydrate of some free carbonyl groups. That the carbohydrate present in these structures is not an acidic or sulphated mucopolysaccharide seems probable from the observations that the membranes neither stain metachromatically with toluidin blue nor exhibit a strong degree of basophilia with methylene blue. In this respect our observations differ from those of Bélanger (1953) who states that when stained with toluidin blue these membranes exhibit metachromasia, whereas the 'Chèvremont and Frédéricq reaction for the sulph-hydril groups of keratin was negative'. These findings, Bélanger concludes, point to the presence of 'sulphomucopolysaccharides' in these membranes. Our own observations are contrary to his, in that we have not observed metachromasia but have obtained a moderate reaction for protein-bound disulphide groups, the latter especially in the tectorial membrane. Parenthetically it should be noted that in agreement with Bélanger we also failed to find any reaction by Chèvremont and Frédéricq's method in the tectorial membrane of a rat's internal ear which we had occasion to examine. In commenting on these differences, it should be pointed out that the Barrnett and Seligman reaction for sulph-hydril and disulphide groups is a specific histochemical test, whereas the nature of Chèvremont and Frédéricq's Prussian blue reaction is unknown. Moreover, metachromasia may under some conditions of fixation be produced by substances other than acid mucopolysaccharides (Wislocki, Bunting & Dempsey, 1947), a circumstance possibly accounting for Bélanger's positive finding, since he used both formaldehyde and alcohol.

Although disulphide groups are especially common in keratins, it would be hazardous, without further study of the protein present in these otic structures, to conclude that it was keratinous in nature. It should, however, be noted in passing that Hardesty (1908), in his study of the tectorial membrane of the pig, states that it possesses 'a hyaline matrix, probably keratin'. He gives no substantiating evidence for his conjecture, and in the comprehensive chapters of Kolmer (1927) and Shambaugh (1932) on the structure of the internal ear we find no mention of keratin. In conclusion, although we agree with Bélanger that there is sulphur in these membranous structures, we believe that it is in the form of disulphides of cystine rather than as sulphates of an acid mucopolysaccharide.

From our observations we would conclude that these membranes contain a substance of protein nature which is characterized by its selective staining with alum-haematoxylin and aldehyde fuchsin, by a strong periodic acid-Schiff reaction indicative of a mucopolysaccharide or glycoprotein, and by the possession of disulphide groups associated with cystine. Moreover, it does not stain characteristically with the ammoniacal silver nitrate method for collagenous reticulum or with Weigert's resorcin-fuchsin stain for elastic tissue.

Of further interest is the observation that in the eye and brain there are structures which stain almost identically. In the eye the ciliary zonula reacts similarly (Wislocki, 1952), and in the brain Reissner's fibre which arises from the subcommissural organ (Wislocki & Leduc, 1952*a, b*, 1954; Bargmann & Schiebler, 1952), and the Herring substance of the hypothalamus and neurohypophysis (Bargmann, 1949; Bargmann & Scharrer, 1951; Barnett & Seligman, 1953) exhibit almost identical staining properties. Thus a substance possessing a distinctive constitution appears to characterize these several neural structures. The Herring material of the hypothalamus is believed to be involved in neurosecretory processes, whereas the ocular zonula and the respective membranous structures of the labyrinth are regarded as subserving purely mechanical functions. The functional role of the subcommissural organ and Reissner's fibre has been the subject of much inconclusive speculation. It is noteworthy, however, that Kolmer (1921) called attention to some anatomical similarities between Reissner's fibre, the tectorial membrane and the cupulae of the labyrinth. He drew attention to what he regarded as their similar modes of formation as well as to other histological resemblances, pronouncing them to be 'cuticular structures'.

Bargmann & Schiebler (1952), in discussing the selective staining of Reissner's fibre with alum-haematoxylin, qualify its staining by saying that after all there are various other cells and elements in the body which are similarly stained, for example elastic tissue and the beta granules of the pancreatic islets. They overlook, however, that the two tissue elements which they have selected react quite differently in some other respects from the particular neural substance in question. Thus, elastic tissue stains deeply with Weigert's resorcin-fuchsin stain and by Unna's orcein technique which are reported as being negative in the case of Reissner's fibre (Nicholls, 1912; Wislocki & Leduc, 1952*b*), while the beta granules of the islets are periodic acid-Schiff negative (Halmi & Davies, 1953).

In this connexion it should be noted that none of the neural structures under consideration reacts with orcein or resorcin-fuchsin, if one ignores faint and atypical staining of some Herring bodies and of the tectorial membrane. Although the substance of these structures does not stain with resorcin-fuchsin or orcein and hence is not identical with the substance of elastic tissue, the fact that both substances do stain with alum-haematoxylin and aldehyde fuchsin bespeaks a certain similarity, the chemical basis of which is completely obscure.

The neural structures in question apparently contain disulphide groups ranging from a faint reaction in the case of the otolithic membranes and cupulae to moderately strong staining of the Herring substance, the secretion of the subcommissural organ (Wislocki & Leduc, 1954), and the tectorial membrane. The ciliary zonula has not been examined.

Using aldehyde fuchsin, Halmi & Davies (1953) list all of the substances in the body, known to them, which it stains. These they subdivide into groups on the basis of whether or not they also exhibit metachromasia and give a periodic acid-Schiff reaction. The category which interests us is the one which lists tissues which stain with aldehyde fuchsin, react orthochromatically with toluidin blue and give a positive periodic acid-Schiff reaction. Amongst the substances so characterized, they list epithelial mucoid of some cells of the gastrointestinal tract, gastric chief cell

cytoplasm, lipofuscin pigment in various cells, thyroid colloid, beta granules of the anterior pituitary, some adenohypophyseal colloid and neurohypophyseal neurosecretory substance (Herring material).

Although the substances which they have listed react similarly with the three stains which they used, the group is disparate on other grounds, with most of its members differing from the neural substances under discussion. In contrast to the substance of the neural structures, lipofuscin pigment would at once be ruled out, for besides its being coloured and giving sundry lipid reactions, it reacts negatively for disulphides. The beta granules of the anterior pituitary cells are negative for disulphides (Ladman & Barnett, 1954). The thyroid colloid, in its intense staining with both acid and basic dyes and its exceptionally strong reaction for disulphide groups (Barnett & Seligman, 1954), would also seem to differ somewhat from the neural substance. Similarly, the colloid of the hypophyseal residual lumen reacts more strongly for disulphide groups than the neural protein in question. We have no comment to offer about the intestinal mucoid cells and the gastric chief cells. From these considerations it seems likely that the substance of the group of structures in brain, eye and inner ear differs in some respects from the majority of the similar, non-neural materials listed by Halmi & Davies.

Despite the close similarities of the histological and histochemical reactions of the neural structures considered in this study, it should be pointed out in conclusion that they are not absolutely identical. For example, the reaction for disulphide groups is much stronger in the Herring substance, the tectorial membrane and the secretion of the subcommissural organ than in the substance of the otolithic membranes and cupulae. Furthermore, the Schiff reaction without previous oxidation is stronger in the Herring substance and tectorial membrane than in Reissner's fibre (Bargmann & Schiebler, 1952), and is absent in the otolithic membranes and cupulae.

#### SUMMARY

The tectorial and otolithic membranes and the cupulae of the internal ear have similar histological and histochemical properties. These structures contain a substance of protein nature which is characterized by its selective staining with alumhaematoxylin and aldehyde fuchsin (Gomori's methods), by a strong periodic acid-Schiff reaction, and by the presence of disulphide groups (Barnett & Seligman's method) associated with cystine. The positive periodic acid-Schiff reaction indicates the presence of a mucopolysaccharide or glycoprotein, but the absence of metachromasia with toluidin blue and of basophilic staining with methylene blue at low pH militate against its being a sulphated mucopolysaccharide.

The selective reactions of these otic structures by the methods cited relate them to several neural structures which exhibit similar staining properties. These are the ciliary zonula, the secretion of the subcommissural organ of the epithalamus, Reissner's fibre, and the Herring substance of the neurohypophysis. These structures are more closely allied to one another with respect to their staining properties than to other substances of the body (e.g. elastic tissue, mucus), from which they can be shown to differ in one or more important ways. With regard to function, there is no apparent similarity between the structures: the several otic membranes in question and the ocular zonula are believed to exercise mechanical functions and the



Herring material to bear a relationship to neurosecretory processes, while the functions of the subcommissural organ and of Reissner's fibre are unknown.

This study was aided by a grant from the Eugene Higgins Trust of Harvard University.

The authors wish to express their appreciation to Mr Arthur Mitchell for preparing the material and to Miss Etta Piotti for the coloured illustrations.

#### REFERENCES

- BARGMANN, W. (1949). Über die neurosekretorische Verknüpfung von Hypothalamus und Hypophyse. *Klin. Wschr.* **27**, 617-622.
- BARGMANN, W. & SCHARRE, E. (1951). The site of origin of the hormones of the posterior pituitary. *Amer. Scientist*, **39**, 255-259.
- BARGMANN, W. & SCHIEBLER, T. H. (1952). Histologische und cytochemische Untersuchungen am Subkommissuralorgan von Säugern. *Z. Zellforsch.* **37**, 582-596.
- BARNETT, R. J. & SELIGMAN, A. M. (1952). Histochemical demonstration of protein-bound sulfhydryl groups. *Science*, **116**, 323-327.
- BARNETT, R. J. & SELIGMAN, A. M. (1953). Investigations of the histochemical localization of disulfides. *J. Histochem. Cytochem.* **1**, 392-393 (Abstract).
- BARNETT, R. J. & SELIGMAN, A. M. (1954). Histochemical demonstration of sulfhydryl and disulfide groups of protein. *J. nat. Cancer Inst.* **14**, 769-803.
- BÉLANGER, L. F. (1953). Autoradiographic detection of  $S^{35}$  in the membranes of the inner ear of the rat. *Science*, **118**, 520-521.
- DEMPSEY, E. W., BUNTING, H., SINGER, M. & WISLOCKI, G. B. (1947). The dye-binding capacity and other chemohistological properties of mammalian mucopolysaccharides. *Anat. Rec.* **98**, 417-430.
- GOMORI, G. (1941). Observations with differential stains on human islets of Langerhans. *Amer. J. Path.* **17**, 395-406.
- HALMI, N. S. (1952). Differentiation of two types of basophils in the adenohypophysis of the rat and the mouse. *Stain Tech.* **27**, 61-64.
- HALMI, N. S. & DAVIES, J. (1953). Comparison of aldehyde fuchsin staining, metachromasia and periodic acid-Schiff reactivity of various tissues. *J. Histochem. Cytochem.* **1**, 447-459.
- HARDESTY, I. (1908). On the nature of the tectorial membrane and its probable role in the anatomy of hearing. *Amer. J. Anat.* **8**, 109-179.
- HOLMGREN, H. & WILANDER, O. (1937). Beitrag zur Kenntnis der Chemie und Funktion der Ehrlichschen Mastzellen. *Z. mikr.-anat. Forsch.* **42**, 242-278.
- HOTCHKISS, R. D. (1948). A microchemical reaction resulting in the staining of polysaccharide structures in fixed tissue preparations. *Arch. Biochem.* **16**, 131-141.
- KOLMER, W. (1921). Das Sagittalorgan der Wirbeltiere. *Z. ges. Anat. 1. Z. Anat. EntwGesch.* **60**, 652-717.
- KOLMER, W. (1927). Gehörorgan. In von Möllendorff's *Handbuch d. mikr. Anat. d. Menschen*, III/1, 250-478. Berlin: Springer.
- LADMAN, A. J. & BARNETT, R. J. (1954). Histochemical demonstration of protein-bound sulfhydryl and disulfide groups in cells of the anterior pituitary. *Endocrinology*, **54**, 355-360.
- LEBLOND, C. P. (1950). Distribution of periodic acid-reactive carbohydrates in the adult rat. *Amer. J. Anat.* **86**, 1-49.
- McMANUS, J. F. A. (1946). Histological demonstration of mucin after periodic acid. *Nature, Lond.*, **158**, 202.
- MITCHELL, A. J. & WISLOCKI, G. B. (1944). Selective staining of glycogen by ammoniacal silver nitrate: a new method. *Anat. Rec.* **90**, 261-266.
- NICHOLLS, G. E. (1912). The structure and development of Reissner's fibre and the subcommissural organ. Part I. *Quart. J. micr. Sci.* **58**, 1-116.
- SHAMBAUGH, G. E. (1932). Cytology of the internal ear. In *Special Cytology*, **3**, 1334-1367. Edited by E. V. Cowdry.
- WISLOCKI, G. B. (1952). The anterior segment of the eye of the rhesus monkey investigated by histochemical means. *Amer. J. Anat.* **91**, 233-262.

- WISLOCKI, G. B. & LADMAN, A. J. (1954). Selective staining of the otolithic membranes, cupulae and tectorial membrane of the inner ear. *Anat. Rec.* (Abstract), **118**, 416.
- WISLOCKI, G. B. & LEDUC, E. H. (1952*a*). Vital staining of the hematoencephalic barrier by silver nitrate and trypan blue, and cytological comparisons of the neurohypophysis, pineal body, area postrema, intercolumnar tubercle and supraoptic crest. *J. comp. Neurol.* **96**, 371-413.
- WISLOCKI, G. B. & LEDUC, E. H. (1952*b*). The cytology and histochemistry of the subcommissural organ and Reissner's fiber in rodents. *J. comp. Neurol.* **97**, 515-544.
- WISLOCKI, G. B. & LEDUC, E. H. (1954). The cytology of the subcommissural organ, Reissner's fiber, periventricular glial cells and posterior collicular recess of the rat's brain. *J. comp. Neurol.* (In the Press).
- WISLOCKI, G. B., BUNTING, H. & DEMPSEY, E. W. (1947). Metachromasia in mammalian tissues and its relationship to mucopolysaccharides. *Amer. J. Anat.* **81**, 1-38.

## EXPLANATION OF PLATES

### PLATE 1

- Fig. 1. Low-power photomicrograph of the internal ear of a newborn mouse stained by Gomori's chrome alum-haematoxylin and phloxine method. The three areas delineated by rectangles contain respectively a crista ampullaris, the macula of the saccule and a portion of the developing organ of Corti. In Pl. 3, figs. 14, 18 and 19, these areas are shown at higher magnifications.  $\times 40$ .
- Fig. 2. A crista ampullaris surmounted by its cupula. Newborn mouse. Chrome alum-haematoxylin and phloxine stain. Compare with Pl. 3, fig. 19, of a similar section which shows the selective blue staining of the cupula by the haematoxylin component of the stain.  $\times 200$ .
- Fig. 3. The macula of the saccule of a newborn mouse with the otolithic membrane. Chrome alum-haematoxylin and phloxine stain. Observe the darkly stained otolithic membrane and compare it with Pl. 3, fig. 14, which illustrates in colour its selective staining.  $\times 200$ .
- Fig. 4. The macula of the utricle of a  $3\frac{1}{2}$ -month-old mouse. Gomori's aldehyde fuchsin stain. Observe the darkly stained otolithic membrane and compare it for detail with a drawing of the same (Pl. 3, fig. 15).  $\times 200$ .
- Fig. 5. A crista of a  $3\frac{1}{2}$ -month-old mouse surmounted by its cupula. Periodic acid-Schiff reaction. Observe the intense coloration of the cupula and compare it with a drawing of the same (Pl. 3, fig. 17).  $\times 200$ .
- Fig. 6. A portion of the cochlea of a newborn mouse showing the developing organ of Corti. Chrome alum-haematoxylin and phloxine stain. Compare with Pl. 3, fig. 18, which shows the selective staining of the developing tectorial membrane in relationship to the underlying cells.  $\times 150$ .

### PLATE 2

- Fig. 7. The organ of Corti of a  $3\frac{1}{2}$ -month-old mouse with the tectorial membrane. Periodic acid-Schiff reaction. Observe the intense staining of the tectorial membrane.  $\times 200$ .
- Fig. 8. The developing organ of Corti of a newborn mouse with the tectorial membrane. Barnett & Seligman's method for disulphide groups. Observe the moderately deep staining of the tectorial membrane, especially of its outer part. The arrow points to the developing hair cells.  $\times 300$ .
- Fig. 9. The developing organ of Corti of a newborn mouse with the tectorial membrane. Weigert's elastic tissue stain. Although appearing relatively dark in the photograph, the staining of the tectorial membrane is atypical, as explained in the text.  $\times 250$ .
- Fig. 10. The developing hair cells of the organ of Corti of a newborn mouse. Method for disulphide groups. Note the moderately strong reaction for disulphide groups in the supranuclear cytoplasm of the hair cells. The arrow points to the tectorial membrane which is also moderately stained.  $\times 650$ .
- Fig. 11. A drawing of the previous field at a higher magnification, showing strongly disulphide-positive hairs developing within the cytoplasm of the hair cells.  $\times 900$ .
- Fig. 12. The developing organ of Corti and the tectorial membrane of a human foetus (17 cm. crown-rump length). Section stained in a methylene-blue solution at pH 4.3. The nuclei are deeply stained at this pH because of the presence of acidic desoxyribonucleoprotein, whereas the tectorial membrane is unstained because of its relative lack of acidity.  $\times 300$ .

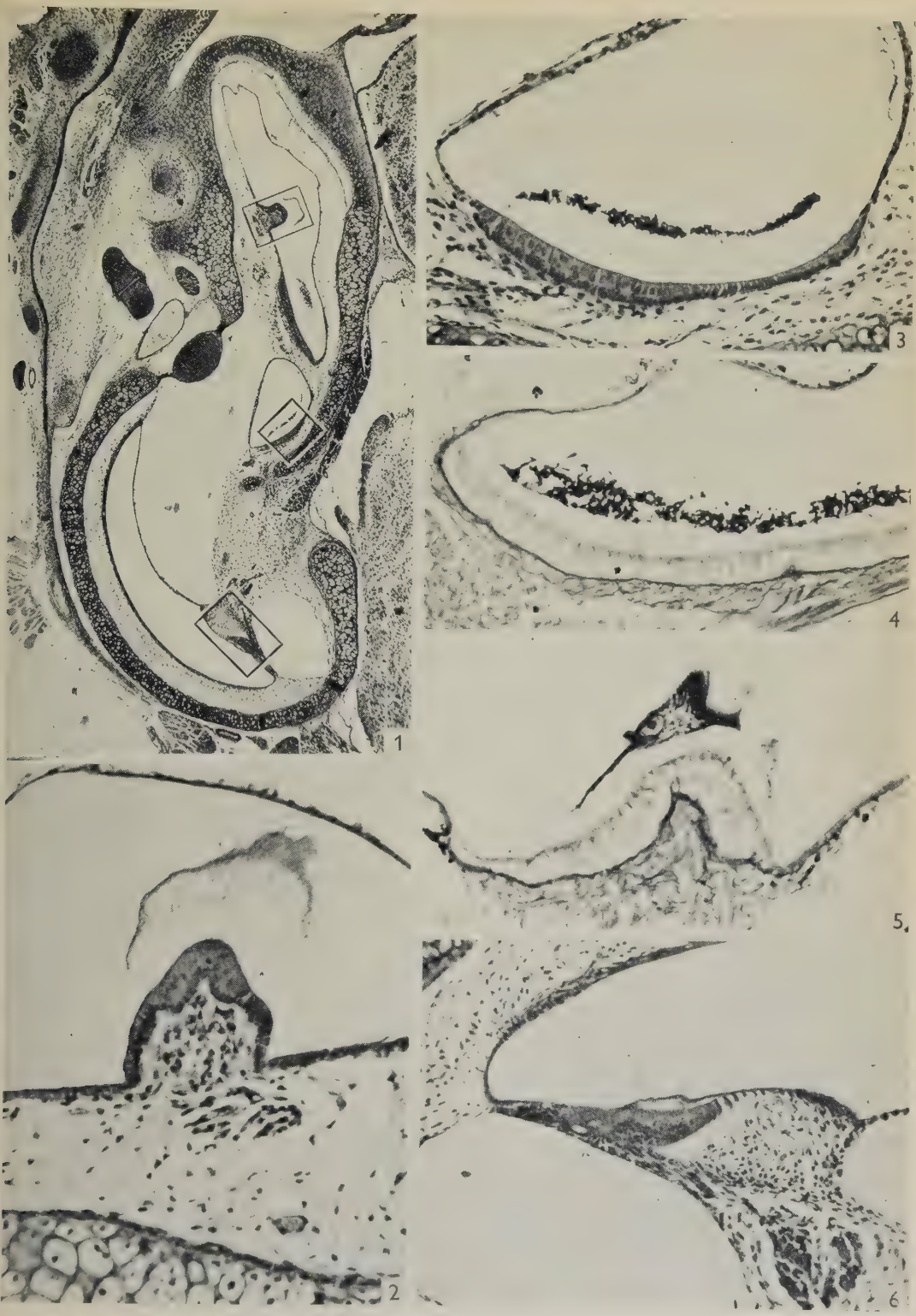


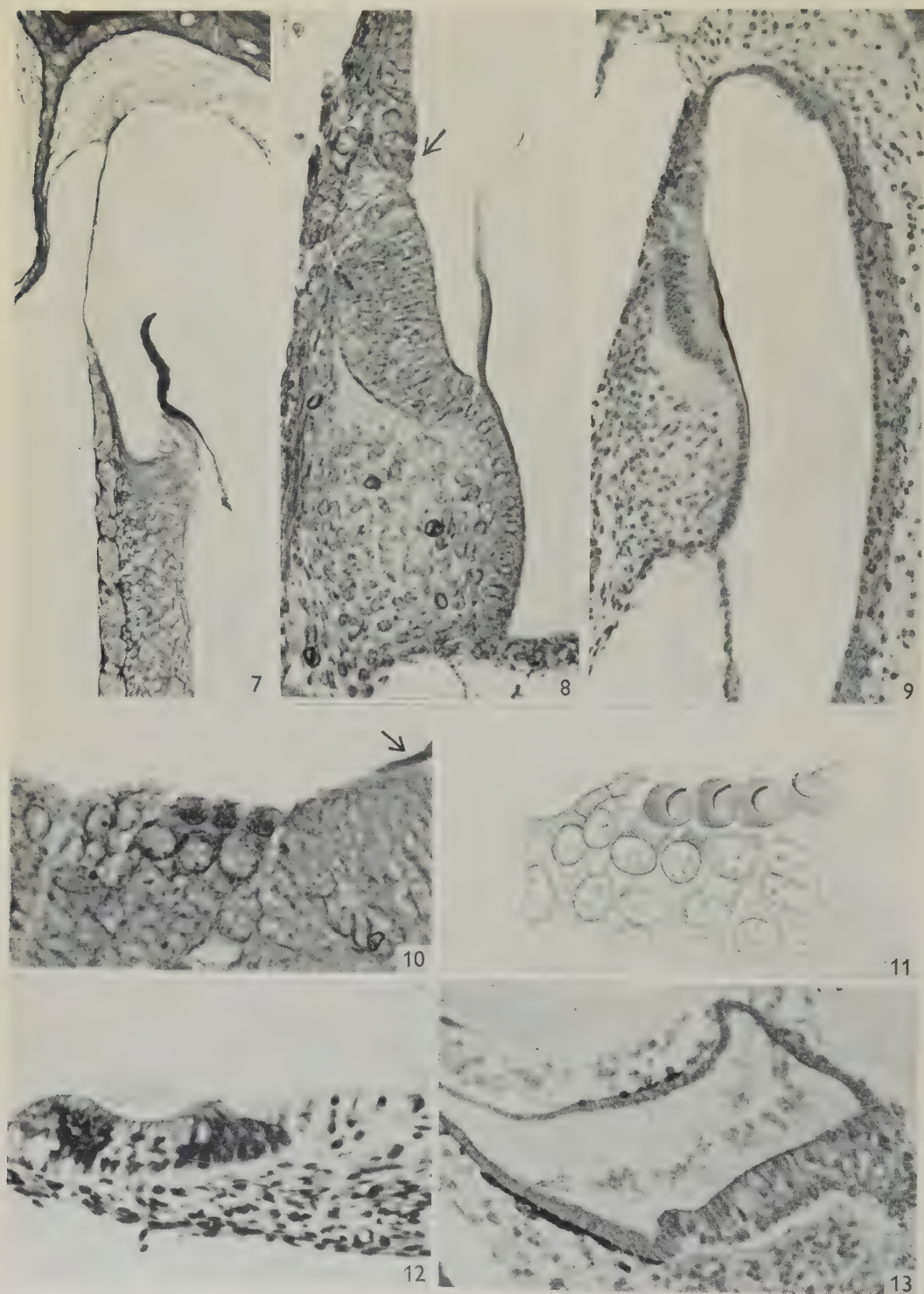
- Fig. 13. A portion of a macula of the utricle of a newborn mouse. Method for disulphide groups. Compare the staining of the distal cytoplasm, hairs and flagella of the sensory epithelium and of the otolithic membrane with the stronger reaction of the tectorial membrane (fig. 8). The black appearance of some parts of the basement membrane beneath the epithelium is due to the presence of melanin.  $\times 300$ .

## PLATE 3

(Figures 14–19 are drawings prepared with a camera lucida)

- Fig. 14. A portion of the macula of the saccule of a newborn mouse, illustrating, in colour, the selective staining of the otolithic membrane by the alum-haematoxylin component of Gomori's stain.  $\times 450$ .
- Fig. 15. A portion of the macula of the utricle of a  $3\frac{1}{2}$ -month-old mouse showing the selective staining of the otolithic membrane by Gomori's aldehyde fuchsin stain.  $\times 800$ .
- Fig. 16. A portion of the macula of the utricle of a  $3\frac{1}{2}$ -month-old mouse, stained by the periodic acid-Schiff method.  $\times 800$ .
- Fig. 17. A cupula with part of a crista ampullaris from the previous specimen, stained by the periodic acid-Schiff method.  $\times 450$ .
- Fig. 18. The organ of Corti of a newborn mouse illustrating the selective staining of the substance of the tectorial membrane by the alum-haematoxylin component of Gomori's stain.  $\times 300$ .
- Fig. 19. A crista ampullaris of a newborn mouse surmounted by its cupula; the latter is stained selectively by the alum-haematoxylin component of Gomori's stain.  $\times 200$ .













# INCORPORATION OF $^{35}\text{S}$ -DL-METHIONINE IN MOUSE TISSUES AS INDICATED BY AUTORADIOGRAPHS

## I. TESTIS, EPIDIDYMIS AND SEMINAL VESICLE

By A. GLUCKSMANN,\* ALMA HOWARD† AND S. R. PELC†

Howard & Pelc (1950) investigated the maturation of cells during spermatogenesis by means of autoradiographs, using  $^{32}\text{P}$  to label the cells. No attempt was made to identify the phosphorus compounds which had taken up  $^{32}\text{P}$ . These timing studies indicated that sperm formation takes about 26 days from the last spermatogonial division to mature sperm. High concentrations of  $^{32}\text{P}$  had to be injected to obtain autoradiographs, and radiation damage, especially slowing down of division during spermatogenesis, could not be excluded. Some of these disadvantages might be avoided by the labelling of desoxyribonucleic acid (DNA) with  $^{14}\text{C}$ -adenine, or of proteins with labelled amino-acids such as methionine. The latter possibility did not at first seem promising because of the expected high turnover of amino-acids in protein as revealed by numerous biochemical studies.

The experiments described in this paper indicate that labelled  $^{35}\text{S}$ -DL-methionine can be used to study the processes of differentiation and secretion in various organs, and that sufficiently stable compounds exist to remain in cells in the testis during maturation.

### MATERIAL AND METHODS

$F_1$  hybrid mice ( $\text{C}_{57}$  black  $\text{♀} \times \text{C}_{3}\text{H}$   $\text{♂}$ ), 4 months old, were given a single intraperitoneal injection of 1.0 ml. of a solution containing 50  $\mu\text{C}$ . of  $^{35}\text{S}$ -DL-methionine in experiments terminated after 2, 4 and 8 hr., and 25  $\mu\text{C}$ . in experiments lasting for longer periods of time. Mice were killed at intervals from 2 hr. to 45 days after injection. Organs were fixed for 1 hr. in acetic acid-alcohol (1:3), then transferred to formol saline (1:8), embedded in paraffin wax and sectioned at 5  $\mu$ . Autoradiographs (ARG) were prepared by the stripping film technique (Doniach & Pelc, 1950), in which a thin photographic film is placed in close contact with the histological section and exposed to the radiation of the isotope. The slide with the section and the film is then put through photographic developer and fixer. This technique allows the section and the photographic film to be viewed simultaneously. Observations were made on unstained preparations with the phase-contrast microscope or on preparations stained with haematoxylin or carmalum. After staining, the slides were dried at room temperature and then mounted directly in Canada balsam.

\* Working with a grant from the British Empire Cancer Campaign at the Strangeways Research Laboratory, Cambridge.

† Experimental Radiopathology Research Unit of the Medical Research Council at Hammer-smith Hospital, London.

## RESULTS

The findings will be discussed under three headings describing the uptake of  $^{35}\text{S}$  derived from labelled methionine in the testis, the epididymis and seminal vesicle. It should be stressed that the autoradiographs show the location of  $^{35}\text{S}$  which need not necessarily be in the form of methionine.

(1) *Testis*. The changes with time in localization of bound  $^{35}\text{S}$  within the seminiferous tubules are indicated diagrammatically in Text-fig. 1. Two hours after the injection of labelled methionine,  $^{35}\text{S}$  is found fairly uniformly distributed over both the cytoplasm and nuclei of the cells in most tubules, though in some it appears to be more concentrated at the periphery than towards the lumen (Pl. 1, fig. 2). After 4 hr. the majority of tubules give a stronger ARG over the outer zone, in which the spermatogonia and primary spermatocytes are situated, than over the more mature inner cells. At this time the peripheral ARG is related to the nuclei of the cells rather than to the cytoplasm. After 8 and 16 hr. ARG appears as a peripheral or outer ring of blackening while it is weak over the inner regions.

These outer rings are still present after 24 hr. though occasionally ARG is seen over groups of cells which project from the outer ring to the lumen in a radial direction. This becomes more evident after 2 days (Pl. 1, fig. 3), though the concentration of grains over the peripheral rings is still stronger than over the radial projections.

After 6 days the ARG over the peripheral parts of the tubules decreases while that over the radial projections increases, and more such projections appear giving a more uniform blackening over the whole cellular part of the tubule. After 8 days (Pl. 2, fig. 4) the cellular part of the seminiferous tubule has a fairly uniform ARG.

The blackening over the peripheral part of the tubule decreases still further by the 12th day, particularly as compared with a zone nearer the lumen which appears as a ring, and which is seen particularly well after 18 days (Pl. 2, fig. 5). The lumen of the tubules usually shows no ARG up to and including this time.

After 26 days ARG is found over mature sperm in the lumen of the tubule, and there are also occasional spermatogonia which are still labelled. The autoradiographs over the testis are very weak after 32 days but show occasionally over some spermatogonia, and by the 45th day they are negligible.

These findings suggest that  $^{35}\text{S}$  introduced as methionine is incorporated into the spermatogonia and primary spermatocytes and concentrated in their nuclei and those of their immediate descendants, forming the 'outer ring' during the first 2 days (Text-fig. 1). As groups of spermatocytes mature, they move towards the lumen in radial sectors up to the 6th day. As their movement proceeds, a uniform blackening of the whole tubule is found; at the same time the ARG of the spermatogonial layers decreases in intensity. This decrease is due to the distribution of  $^{35}\text{S}$  over a greater number of cells by the repeated division of the spermatogonia. As on the other hand the maturing spermatocytes and spermatids retain their  $^{35}\text{S}$  undiluted by further divisions, the 'inner rings' appear and finally are found over the mature sperm in the centre of the lumen itself after 26 days. As will be seen below, the mature sperm retain their  $^{35}\text{S}$  during their voyage through the epididymis.

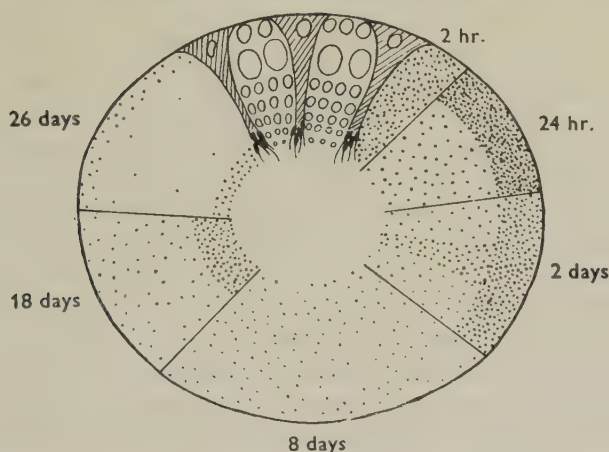
Some of the spermatogonia are still positive after 26 days, which suggests that

they have undergone fewer divisions than those which have handed on most of their  $^{35}\text{S}$  by that time.

The interstitial cells give an ARG as early as 2 hr. and some of them retain it for about 32 days. Particularly during the first 2 days more  $^{35}\text{S}$  is present in the cytoplasm than in the nuclei. Later on nuclear as well as cytoplasmic ARG is found.

The tunica albuginea also takes up  $^{35}\text{S}$  and may retain some of it for 32 days.

(2) *Epididymis*. The epithelium of the epididymis gives a very strong ARG over the cytoplasm of the cells (Pl. 3, fig. 6). This appears to be stronger at the cuticular border from 2 hr. until 2 days at which time  $^{35}\text{S}$  is found only occasionally in the lumen. By the 6th day the amount of  $^{35}\text{S}$  in the lumen increases while that in the epithelium decreases, so that on the 6th and 8th day the concentration of bound  $^{35}\text{S}$  in the epithelium and in the lumen are about equal as judged from the ARG. By the 12th day the ARG over the lumen though weak is stronger than before while the



Text-fig. 1. Diagram of the localization of  $^{35}\text{S}$ -compounds in the mouse testis at different periods after the intraperitoneal injection of  $^{35}\text{S}$ -DL-methionine. The drawings on top indicate spermatogonia, spermatocytes, spermatids, spermatozoa and Sertoli cells. The dots illustrate the localization in the various types of cells of  $^{35}\text{S}$ -compounds giving autoradiographs.

epithelium continues to weaken and becomes negative on the 18th day (Pl. 3, fig. 7). At that time some of the tubules show a definite ARG over the lumen which is stronger than on the 12th day and is frequently associated with collections of sperm (Pl. 3, fig. 7). On the 26th day ARG is found only where mature sperm are present in the lumen. The ARG over the epididymis is negative by the 45th day.

It thus appears that  $^{35}\text{S}$  is first bound in the epithelium of the epididymis and then secreted into the lumen. The autoradiograph in the lumen may, in addition, be due to  $^{35}\text{S}$  being carried down from the testis unassociated with cells during the first 2 days and much later arriving with the mature sperm from the testis.

(3) *Seminal vesicles*. These organs show the most marked uptake of  $^{35}\text{S}$  which can be seen as blackening even with the naked eye. In the mouse this gland has a closely folded epithelium at the fundus (Pl. 4, fig. 8 top)—i.e. at the region furthest removed from the urethral end, and a less folded epithelium surrounding a dilated lumen in the central regions and towards the urethra (Pl. 4, fig. 8 bottom). The



lumina are small in the fundal region, but are large in the central and urethral regions. In all parts the lumina are filled with secretion, but they are found to behave differently in their uptake of labelled methionine.

Though the epithelium and secretion in the lumina show ARG from 2 hr. after injection, there is a distinct gradient: the secretion in the region of the fundus with its small lumina and closely folded epithelium, shows very strong ARG (Pl. 4, fig. 9*a*), while that in the dilated lumina contains far less  $^{35}\text{S}$ . Pl. 4, fig. 9*a*, shows one of the small lumina and folds of a seminal vesicle 8 hr. after the injection of methionine: the lumen shows intense blackening and there is considerable but less uptake of  $^{35}\text{S}$  in the cuticular part of the cells, and still less in the basal region. Pl. 4, fig. 9*b*, gives for comparison cells stained with periodic acid Schiff-reagent which shows a dense basal region and a vacuolated cuticular region of these cells. After 2 days the ARG over the fundus decreases, while that nearer the urethral end increases, and on the 8th day the gradient is actually reversed: there is a stronger ARG over the urethral region than over the fundal region of the seminal vesicle. Up to this time the amount of  $^{35}\text{S}$  is equal in the cytoplasm and the secretion, but from 8 days on it is definitely greater in the secretion. The ARG diminishes in intensity, but is still observable over the secretion after 45 days. The original gradient is concerned with the concentration of  $^{35}\text{S}$  in the lumina rather than in the epithelium, which shows equal ARG over the fundal and urethral regions. The same holds true when the gradient is reversed: the difference is between the amount of bound  $^{35}\text{S}$  in the secretion rather than that in the epithelium.

These gradients in concentration of  $^{35}\text{S}$  can be related to the local ratio of the number of epithelial cells to the volume of the lumen in the various regions of the gland rather than to any differences in the activity of epithelial cells. At the fundus where the lumen is small and the epithelial cells many, the secretion rapidly fills the narrow lumina and is later moved towards the urethra. This rapid secretion and discharge accounts for both the original gradient and its later reversal. The rapid secretion of the labelled product into the narrow lumina accounts for their strong ARG and for the same reason this later on becomes weak as the secretion is diluted by unlabelled material secreted in this region, as well as by the movement of labelled material towards the urethra. The dilated regions of the glandular lumen receive material from all parts and store it for some time. Consequently the autoradiograph here becomes intense more slowly, but on the other hand, persists longer.

#### DISCUSSION

Our autoradiographs indicate the presence of  $^{35}\text{S}$ , derived from the injected  $^{35}\text{S}$ -DL-methionine, in whatever form it may have been incorporated and retained in the sections after fixation, washing, embedding and removal of paraffin-wax. From the known data on the fate of  $^{35}\text{S}$  after injection of labelled methionine, it can be assumed that most of the autoradiographs are due to  $^{35}\text{S}$  incorporated in protein as methionine or cystine, but some may be in other sulphur-containing compounds such as ergothioneine (Mann & Leone, 1953; Heath, Rimington, Glover, Mann & Leone, 1953).

The change of the fairly uniform autoradiograph over the whole of the seminiferous tubules of the testis to the localization in 'outer rings' at 8 hr. indicates the formation of both biologically stable and labile compounds containing  $^{35}\text{S}$ . The

labile compounds or their  $^{35}\text{S}$  are lost either by active turnover in which the labelled material is replaced by unlabelled S-compounds, or by some other metabolic process.

The most likely explanation of our findings on the testis is that in spermatogonia and primary spermatocytes  $^{35}\text{S}$  is incorporated and is retained whilst the cells mature. The timing of spermatogenesis by this method agrees with estimates obtained by various methods (reviewed by Howard & Pelc, 1950). The autoradiographs show  $^{35}\text{S}$  to be incorporated in the nuclei of spermatogonia and primary spermatocytes, which suggests that the portion which is retained by the cells may represent incorporation into the nucleoprotein, although this point can certainly not be regarded as established until more evidence is available. Taylor (1953) and Plaut (1953) found that incorporation of  $^{32}\text{P}$  into DNA during gametogenesis in plants takes place in interphase preceding the first meiotic division and possibly during diplotene. These stages are included in the stages of spermatogenesis during which  $^{35}\text{S}$  in our experiments is taken up. It is hoped to follow this up by experiments on the incorporation of  $^{14}\text{C}$ -adenine into DNA in the mouse testis.

It is of some interest that the interstitial cells of the testis seem to retain their  $^{35}\text{S}$  for a period similar to that of spermatogenesis suggesting some cyclical activity of these cells with a periodicity similar to that of spermatogenesis.

The observations on the epididymis indicate that methionine, or its derivatives, is incorporated into proteins in the cytoplasm of epithelial cells and there retained for about 2 days. It is then slowly discharged into the lumen and disappears.

The very rapid incorporation of  $^{35}\text{S}$  in the secretion of the seminal vesicle, combined with a relatively weak autoradiograph over the epithelial lining, is remarkable. It suggests that the cellular synthesis and secretion of a product incorporating a derivative of methionine is so rapid that only very low concentrations are observed in the cells while the rather more stable secretion allows it to be seen for a longer period in the lumina, where it is also stored. There is also the possibility that the  $^{35}\text{S}$  only passes through the cells and that some of the transformation and incorporation of the methionine takes place in the secretion already in the lumina.

#### SUMMARY

$^{35}\text{S}$ -DL-methionine was injected into mice and the distribution of  $^{35}\text{S}$  in testis, epididymis and seminal vesicles was followed by means of autoradiographs (ARG). Mice were killed from 2 hr. to 45 days after injection.

In the testis  $^{35}\text{S}$  is found in the nuclei of spermatogonia and spermatocytes, and is retained during maturation. Timing of spermatogenesis, based on the movement of  $^{35}\text{S}$ , leads to a figure of 26 days which is in agreement with previous estimates. Some spermatogonia retain appreciable amounts of  $^{35}\text{S}$  for 32 days, indicating that they did not divide as frequently as other spermatogonia. The interstitial cells of the testis retain  $^{35}\text{S}$  in nuclei and cytoplasm for about 26–32 days, i.e. the period of spermatogenesis.

In the epididymis labelled methionine or its derivatives, appears at first in the cytoplasm of the epithelial cells and is later secreted into the lumina. From the 18th day onwards positive autoradiographs in the lumen of the epididymis are associated with collections of sperm.

The seminal vesicles show a very strong uptake of  $^{35}\text{S}$ -DL-methionine or its derivatives at first in the lumina of the closely folded fundus region and later in the larger lumina of the central and urethral regions. The uptake in the cells of the different parts of the gland is the same per cell and the difference in the intensity of the ARG over the secretion in different parts of the gland at different times can be related to the local ratio of number of epithelial cells to volume of the lumen and to the discharge of the secretion in the direction of the urethra.

We wish to record our thanks to Dr H. B. Fell, F.R.S., and to Dr G. Popjak for helpful criticisms of the manuscript.

#### REFERENCES

- DONIACH, I. & PELC, S. R. (1950). Autoradiograph technique. *Brit. J. Radiol.* **23**, 184–192.  
 HEATH, H., RIMINGTON, C., GLOVER, T., MANN, T. & LEONE, E. (1953). Studies using radioactive sulphur on ergothioneine formation in the pig. *Biochem. J.* **54**, 606–611.  
 HOWARD, A. & PELC, S. R. (1950).  $\text{P}^{32}$  autoradiographs of mouse testis. *Brit. J. Radiol.* **23**, 634–641.  
 MANN, T. & LEONE, E. (1953). Studies on the metabolism of semen. *Biochem. J.* **53**, 140–148.  
 PLAUT, W. S. (1953). DNA synthesis in the microsporocytes of *Lilium Henryi*. *Hereditas*, **39**, 438–444.  
 TAYLOR, J. H. (1953). Autoradiographic detection of incorporation of  $\text{P}^{32}$  into chromosomes during meiosis and mitosis. *Exp. Cell Res.* **4**, 164–173.

#### EXPLANATION OF PLATES

##### PLATE 1

- Fig. 2. A seminiferous tubule of the testis seen in (a) a phase-contrast-photomicrograph and (b) an autoradiograph 2 hr. after the intraperitoneal injection of  $50\ \mu\text{C}$  of labelled methionine.  $\times 250$ .  
 Fig. 3. A seminiferous tubule of the testis seen in (a) a phase-contrast-photomicrograph and (b) an autoradiograph 2 days after the intraperitoneal injection of  $25\ \mu\text{C}$  of labelled methionine.  $\times 250$ .

##### PLATE 2

- Fig. 4. A seminiferous tubule of the testis seen in (a) a phase-contrast-photomicrograph and (b) an autoradiograph 8 days after the intraperitoneal injection of  $25\ \mu\text{C}$  of labelled methionine.  $\times 250$ .  
 Fig. 5. A seminiferous tubule of the testis seen in (a) a phase-contrast-photomicrograph and (b) an autoradiograph 18 days after the intraperitoneal injection of  $25\ \mu\text{C}$  of labelled methionine.  $\times 250$ .

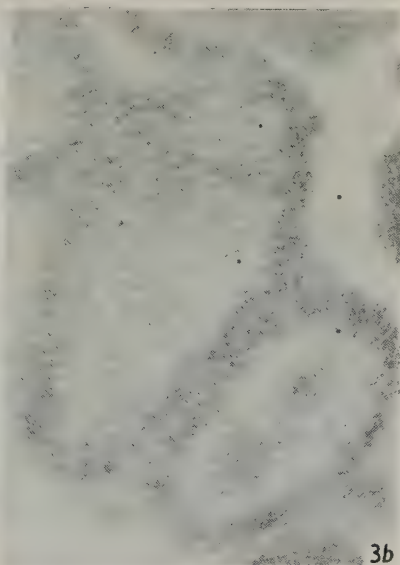
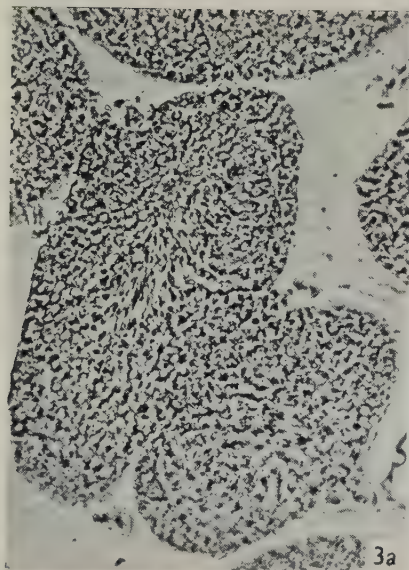
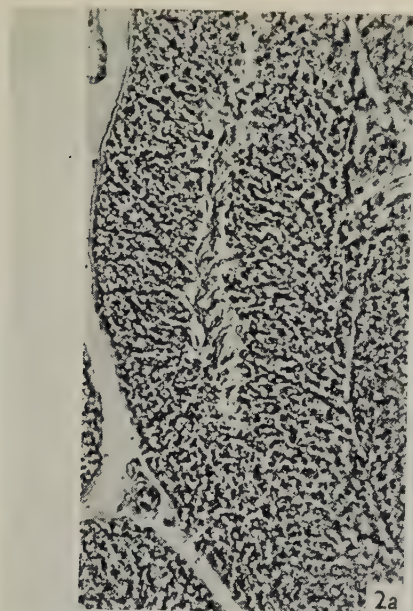
##### PLATE 3

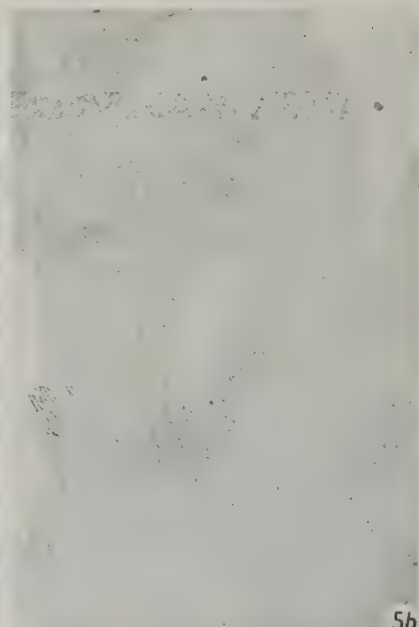
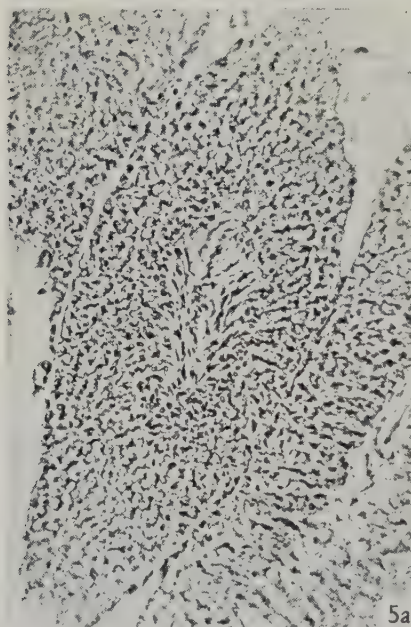
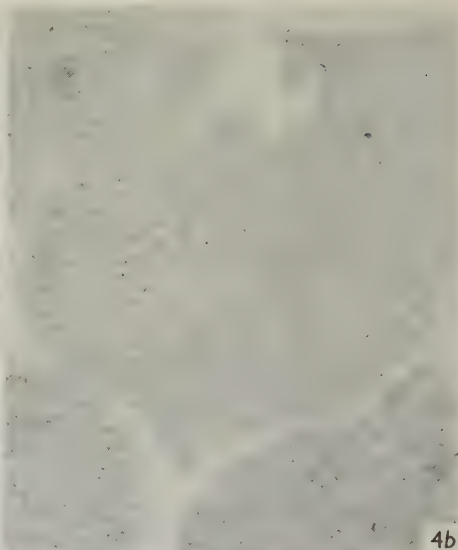
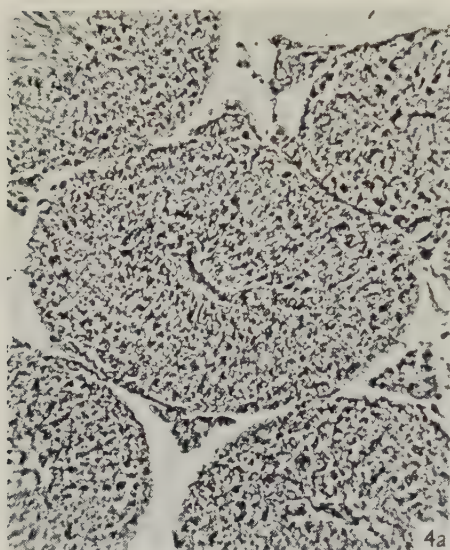
- Fig. 6. Tubules of the epididymis seen in (a) a phase-contrast-photomicrograph and (b) an autoradiograph 1 day after the intraperitoneal injection of  $25\ \mu\text{C}$  of labelled methionine.  $\times 250$ .  
 Fig. 7. Tubules of the epididymis seen in (a) a phase-contrast-photomicrograph and (b) an autoradiograph 18 days after the intraperitoneal injection of  $25\ \mu\text{C}$  of labelled methionine.  $\times 250$ .

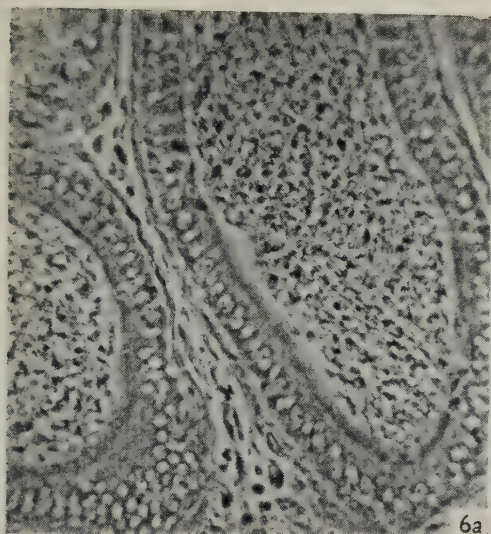
##### PLATE 4

- Fig. 8. The fundus (top) and central region (bottom) of a seminal vesicle seen in (a) a phase-contrast-photomicrograph and (b) an autoradiograph 2 hr. after the intraperitoneal injection of  $50\ \mu\text{C}$  of labelled methionine.  $\times 27$ .  
 Fig. 9. The fundus region of a seminal vesicle 8 hr. after the intraperitoneal injection of  $50\ \mu\text{C}$  of labelled methionine in (a) an autoradiograph stained with carmalum ( $\times 600$ ), and (b) a photomicrograph of a section stained with the Schiff-reagent after treatment with periodic acid.  $\times 1800$ .





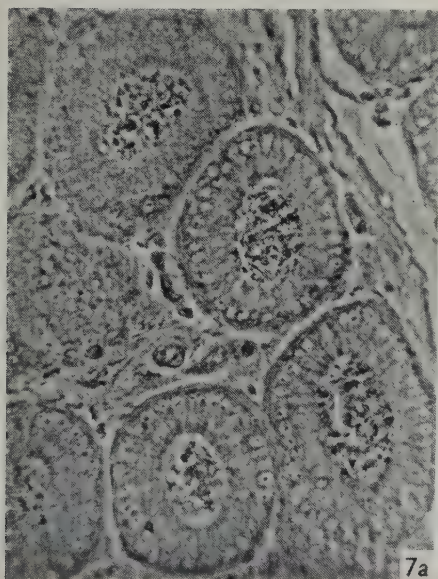




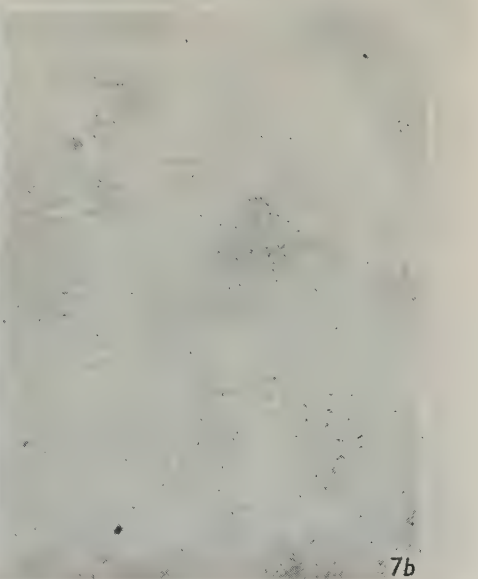
6a



6b

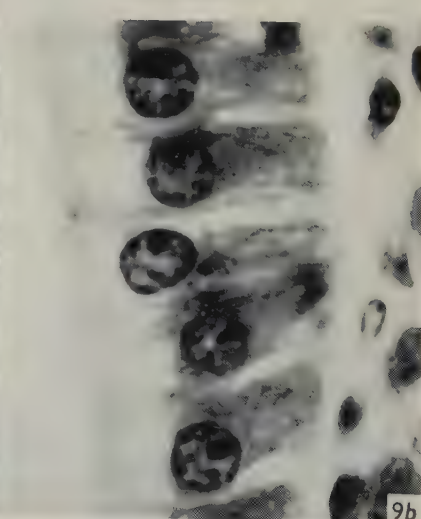
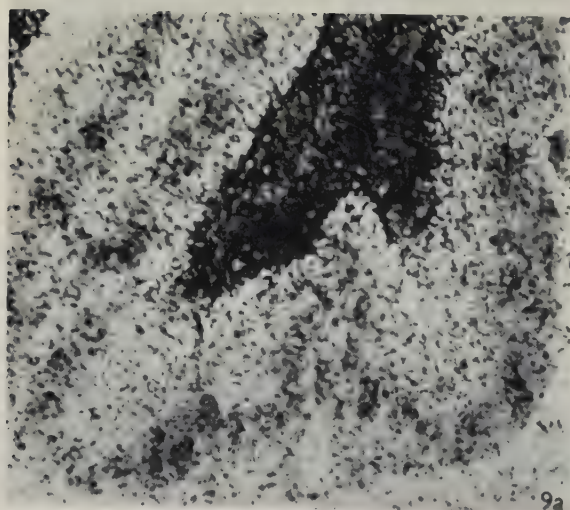
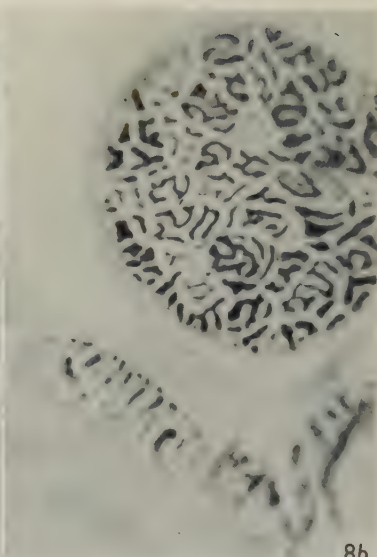
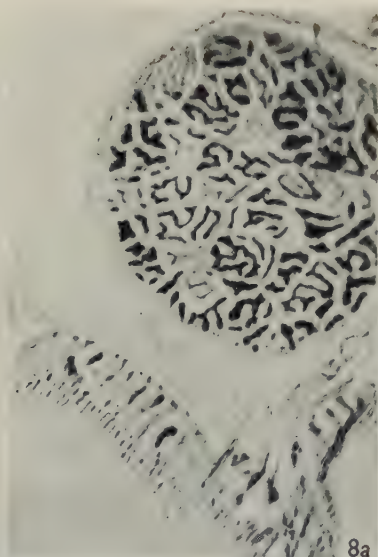


7a



7b





GLUCKSMANN, HOWARD AND PELC—INCORPORATION OF  $^{35}\text{S}$ -DL-METHIONINE IN MOUSE TISSUES

# A QUANTITATIVE STUDY OF THE MAMILLARY BODIES AND THEIR CONNEXIONS

By R. W. GUILLERY\*

*Department of Anatomy, University College, London*

## INTRODUCTION

The mamillary bodies are comparatively small and clearly defined groups of cells where the impulses from a number of widely separated parts of the brain meet and presumably interact. They form a part of the hypothalamus, and they are linked by their afferent and efferent tracts to the anterior thalamus, the hippocampus and the midbrain tegmentum. Following Gudden's description of the mamillary tracts (Gudden, 1889) a number of detailed studies of the mamillary bodies and their fibre connexions have appeared, but the functional role of the mamillary bodies is not known. The majority of authors have described either the topographical relations of the mamillary tracts, or the size, shape, staining reaction and distribution of the mamillary cells. Quantitative studies have only appeared recently. Rose (1939-40) published some volume measurements of the mamillary nuclei, and Simpson (1952) and Daitz (1953) have counted the number of fibres in the fornix of the macaque and man. Although these quantitative studies have drawn attention to certain inter-species differences in the mamillary system of connexions, they are not sufficient to form any coherent picture of the quantitative aspect of the comparative anatomy of this system. Nor do they show the relative size of each of the mamillary components within any one species. The present counts were planned to give a numerical criterion for assessing the relative importance of the parts of the mamillary system of connexions and to form a basis for a quantitative study of mamillary organization. The cells in the medial and lateral mamillary nuclei and the fibres in the fornix, the mamillary peduncle, the principal mamillary tract† and the mamillo-thalamic tract of the cat and the rabbit have been counted.

## MATERIALS AND METHODS

All the brains that have been used in the present investigation were fixed by perfusion with formol saline and were cut in paraffin. The cells were stained with a 0.25 % aqueous solution of thionin buffered at pH 4.6 with an acetic acid:acetate buffer. The fibres were stained by the Holmes silver method‡ (Holmes, 1947). Some of the series were stained by both the silver and the Nissl method. In these, regularly spaced pairs of adjacent sections were selected and one of each pair was silver stained, the other Nissl stained.

\* This work was carried out during the tenure of a University of London Postgraduate Studentship.

† The principal mamillary tract is the common origin of the mamillo-thalamic and mamillo-tegmental tracts from the mamillary nuclei (Koelliker, 1896).

‡ In stage 2 of the procedure 3 ml. of 1 % aqueous silver nitrate and 5 ml. of pure pyridine were used. In stage 6 the reducer was heated to 50° C.

It has not been possible to obtain a complete set of cell and fibre counts from individual animals. Certain thicknesses and planes of section have been found particularly suitable for each of the counts. Table 1 gives details of sectioning and also shows the animal from which each of the counts has been taken.

Table 1. *The series that have been used for the cell and fibre counts.*

('Oblique' is the plane perpendicular to the direction of the post-commissural fornix (see Text-figs. 1-4). *F*, fornix counts; *LMN*, lateral mamillary cell counts; *MMN*, medial mamillary cell counts; *MP*, mamillary peduncle counts; *PMT*, principal mamillary tract counts; *MT*, mamillo-thalamic tract counts.)

Animal no.	Plane of section	Thickness of section ( $\mu$ )	Staining method	Observations
Cat 1	Frontal	18	Nissl and silver	<i>LMN, MP</i>
Cat 2	Frontal	12	Nissl and silver	<i>LMN, MP</i>
Cat 3a	Oblique	5	Silver	<i>F</i>
Cat 3b	Horizontal	15	Nissl and silver	<i>PMT, MT</i>
Cat 4	Oblique	7	Silver	<i>F</i>
Cat 5	Frontal	17	Nissl and silver	<i>MMN, MP</i>
Cat 6	Horizontal	11	Silver	<i>PMT, MT</i>
Cat 7	Parasagittal	15	Nissl and silver	<i>MMN</i>
Rabbit 1	Frontal	12	Nissl and silver	<i>MP</i>
Rabbit 2	Frontal	18	Nissl and silver	<i>MP, MMN, LMN</i>
Rabbit 3a	Oblique	8	Silver	<i>MP, F</i>
Rabbit 3b	Parasagittal	15	Nissl and silver	<i>MMN, LMN</i>
Rabbit 4	Oblique	8	Silver	<i>F</i>
Rabbit 5	Oblique	6	Silver	<i>MT</i>
Rabbit 6	Horizontal	10	Silver	<i>PMT</i>
Rabbit 7	Horizontal	10	Silver	<i>PMT, MT</i>

### Cell counts

The cells in the medial and lateral mamillary nuclei have been counted. These nuclei can be clearly recognized in the Nissl preparations, and in the silver preparations they have a dense pericellular fibre plexus which distinguishes them from neighbouring cell groups. The supramamillary, premamillary (Rioch, 1929) and paramamillary (Koikegami, 1938) nuclei have a comparatively sparse plexus and have not been included in the present counts. A small group of small cells in the anterior part of the medial nucleus, which shares the dense plexus of this nucleus, has been included in the medial mamillary cell counts. This group probably corresponds to the dorsal premamillary nucleus of Krieg (1932).

The sections for all the counts were taken from complete series at intervals of 12-18 sections. The outline of the medial mamillary nucleus was projected on to squared paper at a constant magnification, each square representing  $150 \times 150 \mu$  of section. In the medial nucleus the nerve-cell nuclei and parts of nuclei were counted in alternate squares. Only the squares that were completely included in the outline were included in the counts. The area of the medial nucleus was found by weighing tracing paper of uniform thickness which had been cut to the shape of the projected outline. From these results it was possible to calculate the total number of medial mamillary cells per section and hence the number of cells in the medial nucleus. In the lateral mamillary nucleus all the cells in any one section were counted since this nucleus is small in both the rabbit and the cat. The correction factor described by Abercrombie (1946) was used in calculating all the totals. In each of the cell counts between 1.5 and 2.5% of the total number of cells was counted.



*Fibre counts*

The section of the tract that was to be counted was drawn on to squared paper as above, and the area occupied by the tract was found by the same method. The boundaries of the principal mamillary tract and those of the fornix can be distinguished clearly, but those of the mamillo-thalamic tract and of the mamillary peduncle are less distinct. The error introduced in measuring the area of the mamillary peduncle may be as high as 15 %, that introduced in measuring the area of the mamillo-thalamic tract is slightly lower. The fibres were counted in square fields  $4.5 \times 4.5 \mu$ . Between 100 and 400 such fields, selected from all parts of the tract by means of a table of random numbers, were counted in each section. The number of fibres counted was between 1 and 5 % of the total.

Standard deviations of the individual counts have not been given. These would be misleading since neither the cells nor the fibres are evenly distributed throughout the section but occur in groups of varying density. Furthermore, such a measurement of scatter would not include the sampling error due to the measurement of area.

Four of the fornix counts were taken from pairs of closely adjacent sections. The difference between the members of these pairs (Table 2) is less than 10 %. The similarity of these counts and the similarity of the pattern that has been found in the fornix counts of different individuals of the same species (see Text-figs. 1-4), shows that the error of the fornix counts is probably less than 10 %. The errors of the other fibre counts are of the same order.

RESULTS

*The post-commissural fornix*

The results of the fornix counts are shown in Table 2. The sections for these counts were taken from a complete series and therefore it has been possible to give their spacing along the course of the post-commissural fornix. The results are shown diagrammatically in Text-figs. 1-4. In both the rabbit and the cat there is a large fall in fibre number between the anterior commissure and the midtuberal region. In the rabbit this is about 100,000 fibres, that is, about one-half of the post-commissural fornix; in the cat it is about 60,000-70,000 fibres, that is, about one-third of the post-commissural fornix. In the cat there is a secondary premamillary increase of about 20,000-25,000 fibres; no such increase was found in the rabbit.

It has not been possible to find the origin of the fibres that are responsible for the secondary increase in the cat. Sections prepared by the rapid Golgi method show that some of the individual fornix fibres branch at the level of the mamillary bodies and the premamillary increase may be due to similar branching at more rostral levels. Parasagittal silver preparations show a small group of fibres curving postero-dorsally from the ventro-median hypothalamic nucleus towards the premamillary fornix. These fibres may also contribute to the premamillary increase. Further observations are necessary to show the precise origin of the 20,000-25,000 fibres that appear in the posterior parts of the cat's fornix.

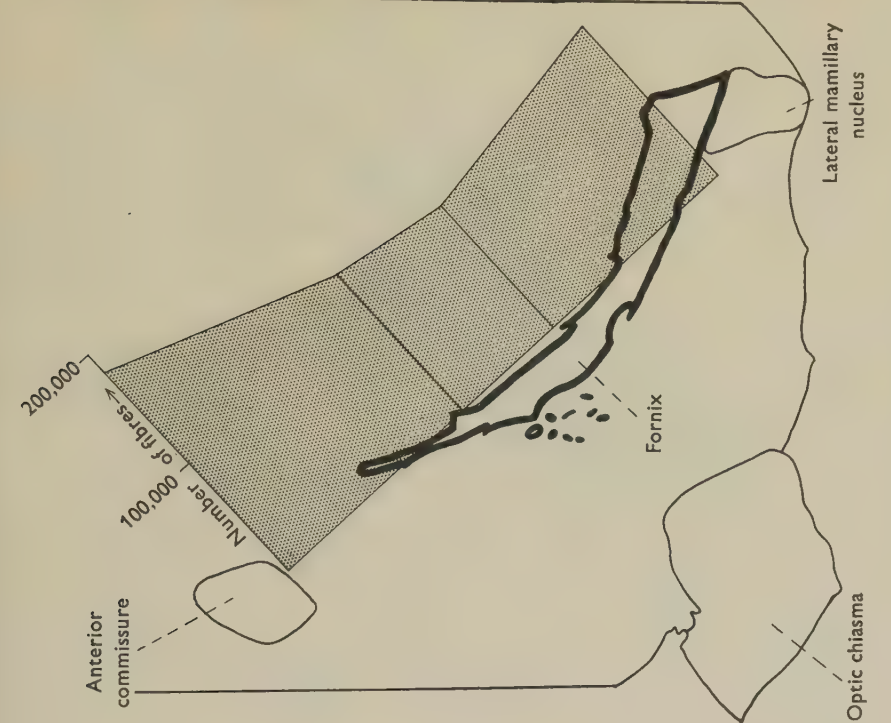
In the anterior part of the hypothalamus, where the fibre loss occurs, a number of fibre bundles can be seen passing from the fornix into the hypothalamus. A small

group of fibres leaves the anterior part of the post-commissural fornix of the rabbit and the cat, to pass ventrally in the periventricular system of the anterior hypothalamus and preoptic region. This group, the medial cortico-hypothalamic tract of Gurdjian (1927), is shown in Pl. 1, fig. 1. The greater part of the tract comes from a loosely organized group of fibres, which lies dorso-medial to the fornix at the level of the anterior commissure (*L* in Pl. 1, fig. 1) and was not included in the counts. A small number of compact bundles (*C* in Pl. 1, fig. 1) from the main bundle of the fornix also join the medial cortico-hypothalamic tract. These were included in the counts but cannot account for more than a small proportion of the total fibre loss.

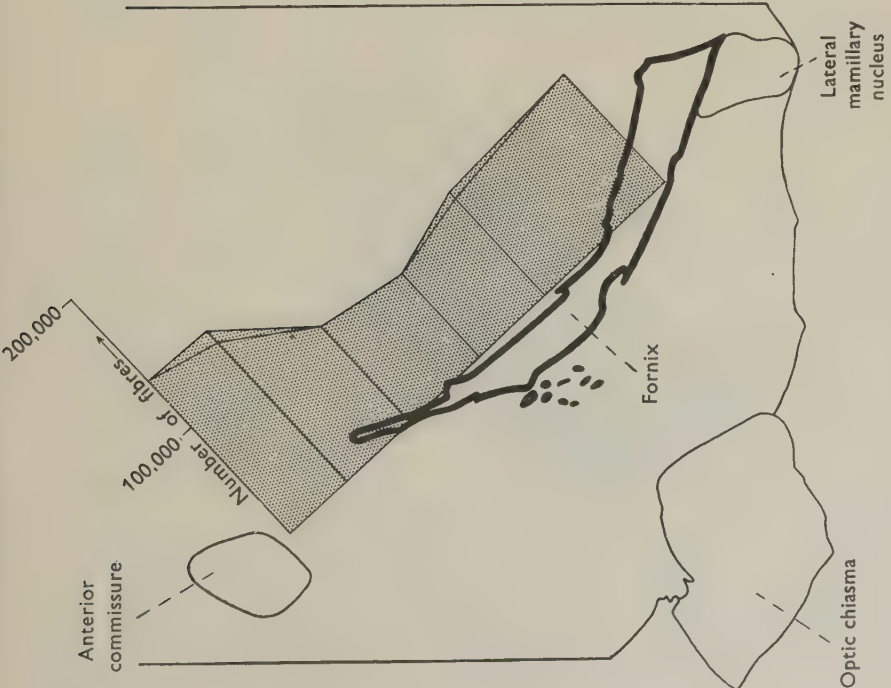
Table 2. *The number of fibres in the parts of the post-commissural fornix*

Distance behind the anterior commissure (expressed as a per- centage of the dis- tance between the anterior commissure and the mamillary bodies) (%)	No. of fibres
Cat 3 ( <i>a</i> )	
2	143,000
{ 15	131,000 }
{ 15	142,000 }
29	89,000
45	78,000
{ 60	90,000 }
{ 60	96,000 }
88	102,000
Cat 4	
1	184,000
40	127,000
61	114,000
99	138,000
Rabbit 3 ( <i>a</i> )	
4	203,000
10	168,000
23	123,000
36	123,000
49	96,000
73	92,000
95	103,000
103	95,000
Rabbit 4	
5	190,000
8	145,000
30	122,000
52	99,000
95	90,000

Two other fibre groups can be seen leaving the anterior parts of the cat's fornix. One leaves the fornix laterally, immediately dorsal to the supraoptic nucleus and passes caudally in the medial forebrain bundle (Pl. 1, fig. 2). Some of these fibres can be followed as far as the mamillary bodies, where they lie ventro-lateral to the lateral mamillary nucleus, but the majority are lost in the lateral hypothalamic nucleus. The second group leaves the fornix medially and passes caudally in the

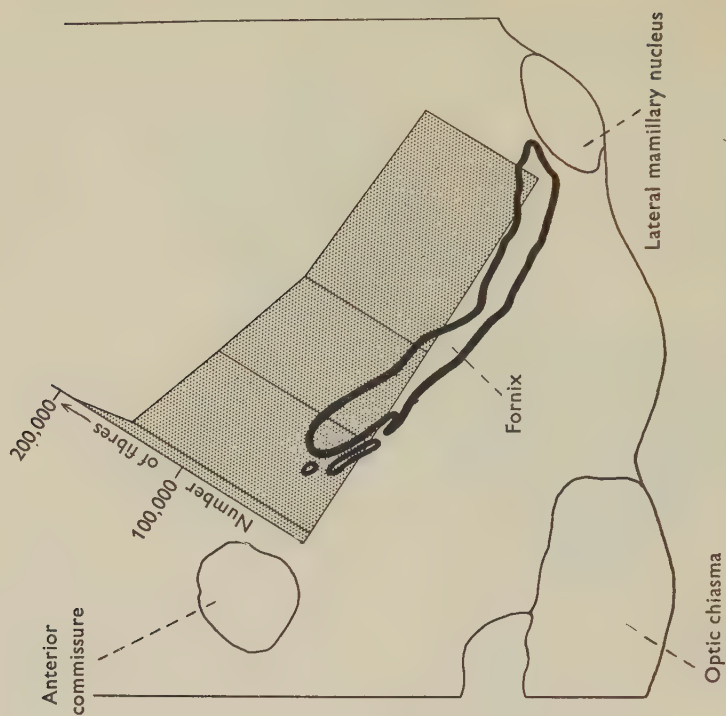


Text-fig. 2. Diagram to show the number of fibres that were counted in the post-commissural fornix of cat 4.

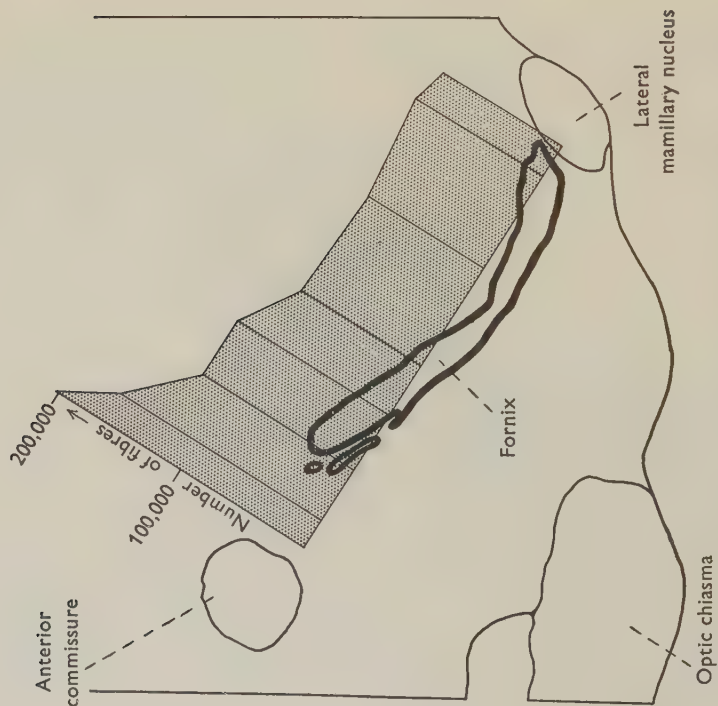


Text-fig. 1. Diagram to show the number of fibres that were counted in the post-commissural fornix of cat 8.





Text-fig. 4. Diagram to show the number of fibres that were counted in the post-commissural fornix of rabbit 4.



Text-fig. 3. Diagram to show the number of fibres that were counted in the post-commissural fornix of rabbit 3.

dorsal part of the hypothalamus (Pl. 1, fig. 3). Some of these fibres can be traced into the posterior hypothalamus almost as far as the principal mamillary tract. It has not been possible to count the number of fibres in either of these groups, or to find their endings. They probably account for a considerable part of the total fibre loss and end among the cells of the premamillary hypothalamus.

No fibres, other than those of the medial cortico-hypothalamic tract, can be seen leaving any part of the rabbit's post-commissural fornix. The great majority of the 100,000 fibres that fail to reach the mamillary bodies must either end close to the fornix, or else leave the main bundle so diffusely that they cannot be seen on normal preparations.

The number of post-commissural fornix fibres that reach the mamillary bodies is about 100,000 in both animals; the number that fail to reach the mamillary bodies is about 100,000 in the rabbit and about 60,000-70,000 in the cat.

*The mamillary cells and the efferent mamillary fibres*

The mamillary cells send their axons into the principal mamillary tract, which divides to form the mamillo-thalamic and mamillo-tegmental tracts. The fibres in the principal mamillary tract and the mamillo-thalamic tract have been counted, but it has not been possible to count the mamillo-tegmental fibres since these mingle with other tegmental fibres immediately caudal to their origin from the principal tract.

The counts of the mamillary cells are shown in the first two columns of Table 3. The total number of mamillary cells is approximately equal to the total number of fornix fibres that reach the mamillary bodies. In the rabbit there are slightly more than 100,000 cells; in the cat there are slightly fewer than 100,000 cells. The lateral mamillary cells form less than 5% of these totals. Although a number of authors (E. G. Rose, 1939-40; Gudden, 1889) have drawn attention to the large size of the rabbit's lateral mamillary nucleus, this nucleus contains fewer cells than that of the cat, both absolutely and in relation to the number of medial mamillary cells. Rose measured the volume of the lateral mamillary nucleus, whereas the number of cells has been recorded here. Pl. 1, figs. 4 and 5 show that the major difference between the lateral mamillary nuclei of the cat, and the rabbit lies in the packing of the cells, the cells in the cat's lateral nucleus being much more closely packed than the cells in the rabbit's lateral nucleus. The greater part of the volume of the rabbit's lateral nucleus is made up of a dense coarse-fibred plexus. Only a relatively small part of

Table 3. *The number of mamillary cells and the number of fibres in the principal mamillary tract*

	No. of mamillary cells		No. of fibres in the principal mamillary tract	
	Medial nucleus	Lateral nucleus	Under 1.5 $\mu$ in diameter	Over 1.5 $\mu$ in diameter
Cat	84,000	3,100	86,000	2,200
	74,000	3,000	77,000	2,900
Rabbit	130,000	2,000	72,000	3,300
	104,000	2,100	79,000	2,600

the cat's lateral nucleus is occupied by such a plexus. The fibres of the mamillary peduncle can be followed into this plexus and, since the calibre of the fibres in the peduncle and in the plexus is approximately the same, it is highly probable that the greater part of this coarse-fibred plexus is made up of mamillary peduncle fibres (*vide infra*).

The medial and lateral mamillary nuclei both send their axons into the principal mamillary tract. Horizontal sections through this tract show that there is a group of particularly coarse fibres in its antero-lateral parts, and these can be followed into the lateral mamillary nucleus in frontal sections. A preliminary study of the distribution of fibre sizes (axon diameter, without myelin) showed that the coarse lateral mamillary efferents are over  $1.5\ \mu$  in diameter, while the fibres in the main part of the tract are under  $1.5\ \mu$ . The two size groups have been recorded separately in Table 3.

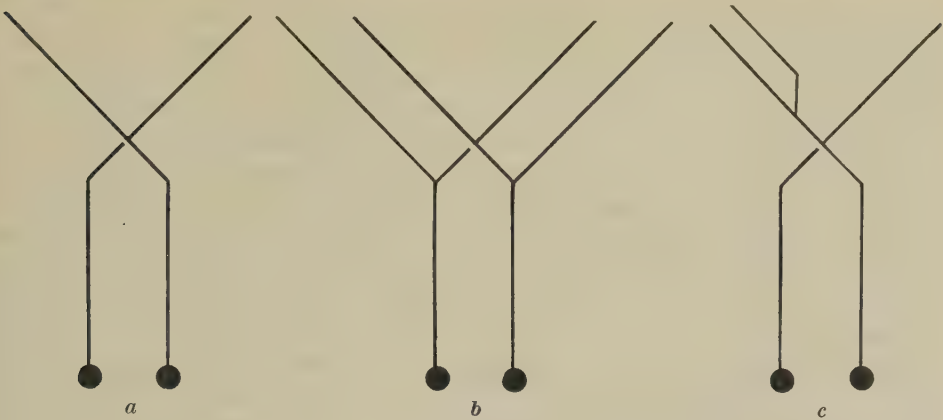
In Table 3 the counts of the mamillary cells have been compared with the counts of the principal mamillary fibres. In both animals the number of lateral mamillary cells shows close agreement with the number of coarse fibres in the principal tract, indicating that each lateral mamillary cell sends one unbranched axon into the principal tract. Similarly, in the cat there is a close agreement between the number of medial mamillary cells and the number of fine fibres in the principal tract. In the rabbit there appear to be fewer fibres in the principal tract than cells in the medial nucleus. The figures suggest that one-quarter or more of the rabbit's medial mamillary cells have axons which either ramify within the medial nucleus or else pass into the medial forebrain bundle. Although the two sets of figures cannot be regarded as conclusive evidence for the presence of such cells they draw attention to a possibility which is of considerable interest.

The principal mamillary tract divides into the mamillo-thalamic and mamillo-tegmental tracts some distance dorsal to the mamillary bodies. It has not been possible to count the number of fibres in the mamillo-tegmental tract, but it is clear from the literature and from the sections that have been examined that a large number of fibres enter this tract from the principal mamillary tract. In both animals the number of fibres in the mamillo-thalamic tract is approximately equal to, certainly not less than, the number of fibres in the principal mamillary tract (Table 4), showing that there must be a considerable amount of branching of the individual fibres of the principal mamillary tract. The present material is not suitable for showing the pattern of this branching. The fibres may branch as shown in Text-fig. 5*b* or as shown in Text-fig. 5*c*. They cannot leave the mamillary bodies without branching (Text-fig. 5*a*).

Table 4. *The number of fibres in the principal mamillary tract and in the mamillo-thalamic tract*

	No. of fibres in the principal mamillary tract	No. of fibres in the mamillo-thalamic tract
Cat	88,000 80,000	115,000 72,000
Rabbit	75,000 82,000	73,000 82,000





Text-fig. 5.

*The mamillary peduncle and post-mamillary fornix*

In the ventro-median parts of the midbrain tegmentum, between the mamillary bodies and the exit of the third cranial nerve, the mamillary peduncle is conspicuous as a coarse-fibred bundle. A considerable number of fine fibres also lie scattered among the characteristic coarse fibres of the peduncle. The coarse fibres can be followed rostrally to the mamillary bodies, where they ramify among the cells of the medial and lateral mamillary nuclei, but it has not been possible to trace the finer fibres on the present material. For this reason the coarse and the fine fibres have been recorded separately in the following counts, fibres over  $1\mu$  in diameter (axon only) being classed as coarse and fibres under  $1\mu$  being classed as fine. The results are given in Table 5, which shows that the coarse fibres form less than a half of the total mamillary peduncle fibres. If all the fibres of the mamillary peduncle reach the mamillary bodies the ratio of fornix afferents to mamillary peduncle afferents is 3:1 or more in the cat, and about 2:1 in the rabbit. If the only coarse fibres reach the mamillary bodies these ratios are as high as 10:1 and 5:1 respectively.

Table 5. *The number of fibres in the mamillary peduncle*

Animal no.	Over $1\mu$ in diameter	Total number of fibres	Medial fornix component
Cat 1	5,000	15,000	—
Cat 2	7,500	18,000	—
Cat 5	12,000	31,500	—
Cat 5	14,000	39,500	—
Rabbit 1	19,000	49,000	11,500
Rabbit 2	17,000	49,000	9,000
Rabbit 3	17,500	55,000	7,000

The counts of cat 5 are considerably higher than those of cats 1 and 2. The mamillary peduncle of the cat 5 occupies an unusually large area but the boundaries of this area are not as definite as they are in cats 1 and 2. It is not possible to assess the significance of this high count until more counts have been done and until the course of the mamillary peduncle fibres is known in more detail. The results from cat 5 make a comparison between the cat and the rabbit material difficult, but the

counts indicate that the mamillary peduncle of the rabbit is larger than that of the cat, suggesting that the mamillary bodies of the rabbit are influenced by tegmental activity to a greater extent than are the mamillary bodies of the cat. These counts also support the view that the dense, coarse-fibred plexus of the rabbit's lateral mamillary nucleus is made up of mamillary peduncle fibres (*vide supra*).

A number of authors have described fornix fibres that pass the mamillary bodies and enter the midbrain tegmentum via the supramamillary commissure (Cajal, 1911; Edinger & Wallenberg, 1902; Morin, 1950) or via the mamillary peduncle (Gerebtzoff, 1941-2; Sprague & Meyer, 1950). On some of the present material (cat as well as rabbit) a few small bundles can be seen passing from the fornix into the supramamillary commissure. It has not been possible to determine either their number or their tegmental course. The fibres that pass into the mamillary peduncle could be identified in the rabbit but not in the cat. The outlines of this group are difficult to define and it is therefore not possible to give an accurate count of the number of fornix fibres in the mamillary peduncle of the rabbit. An approximate estimate has been given in the last column of Table 5. This shows that only about 1/10 of the preamillary fornix fibres pass into the mamillary peduncle and that at least 10,000 of the rabbit's fine mamillary peduncle fibres are not afferent to the mamillary bodies.

#### DISCUSSION

The cell and fibre counts have shown the gross quantitative relations that hold between the mamillary cells, their afferent and their efferent fibres. They have also shown that a large part of the post-commissural fornix does not reach the mamillary bodies and that there is a considerable amount of branching of the individual fibres in the principal mamillary tract.

#### *The post-commissural fornix*

Several authors have described fibres passing from the fornix into the pre-mamillary hypothalamus (e.g. Gudden, 1889, and Edinger & Wallenberg, 1902, for the rabbit; Gurdjian, 1927, for the rat). Daitz (1953) mentions that the fornix gives off small fascicles to the hypothalamus on its way down to the mamillary bodies and Simpson (1952), working on the macaque, found terminal degeneration in the ventro-median hypothalamic nucleus after lesions of the fornix. Simpson, however, points out that his lesions also included the stria terminalis, and that his evidence does not certainly establish a contribution from the fornix to the ventro-median nucleus. Rioch (1931) described the fornix in normal cat material and mentions fibres that leave the fornix through the perifornical nucleus to distribute to the medial preoptic area and to the medial hypothalamic nuclei. Papez (1938) described the brain of a dog in which one hemisphere had been removed, including a part but not the whole of the hippocampus and fornix. He found that the remaining fornix fibres on the affected side did not reach the mamillary bodies but ended in the mid-tuberal region, and he concluded that some of the fornix fibres must end in the hypothalamus.

Although there is good evidence for a hypothalamic ending of fornix fibres, none of the previous evidence shows the size of this fornix component. The present counts show that in the cat about one-third of the post-commissural fornix fibres (60,000-70,000 fibres) fail to reach the mamillary bodies; some of these fibres pass towards the medial, postero-dorsal and lateral parts of the hypothalamus but their precise

end-station is unknown. In the rabbit about one-half of the post-commissural fornix fibres (about 100,000 fibres) fail to reach the mamillary bodies. In the present rabbit material no fibres, other than those of the medial cortico-hypothalamic tract, can be seen leaving the fornix. Sprague & Meyer (1950) found degenerating fornix fibres along the whole length of the perifornical nucleus of the rabbit, but they were unable to find any other fornix degeneration in the hypothalamus. The perifornical endings may, in part, account for the post-commissural fibre loss, but it is doubtful whether as much as one-half of the fornix can end in the perifornical nucleus. If there are fibres passing from the rabbit's fornix into the hypothalamus they must pass diffusely, not in bundles as in the cat, so that their identification in normal or degenerated material would be extremely difficult.

It is probable that the majority of the non-mamillary fibres of the post-commissural fornix end in the hypothalamus. A few pass into the tegmentum. The precise ending of these non-mamillary fibres is not known at present.

The work of Edinger & Wallenberg (1902), Gurdjian (1927) and Krieg (1932) suggests that the hypothalamic component of the fornix arises from the hippocampus as does the mamillary component, but the evidence is not conclusive. Gurdjian describes a bundle of fibres passing from the stria terminalis into the medial cortico-hypothalamic tract, and Morin (1950) describes fibres from the septum that join the post-commissural fornix. It is possible that some of the fibre loss is due to fibres which come from the stria terminalis or the septum, join the post-commissural fornix and leave it again in the premamillary hypothalamus. Further investigations are necessary before the origin and the ending of the non-mamillary component of the fornix can be known in detail, but it is reasonable to assume that at least the greater part of it arises in the hippocampus and ends in the hypothalamus.

#### *The efferent mamillary tracts*

The efferent mamillary fibres link the mamillary bodies with the tegmentum on the one hand, and via the anterior thalamus with the cingulate cortex and thence with the hippocampus (Cajal, 1911; Gardner & Fox, 1948; Adey & Meyer, 1952) on the other. Papez (1937) has drawn attention to the circuit that connects the hippocampus, the mamillary bodies, the anterior thalamus and the cingulate cortex. The activity of this circuit is brought into relation with tegmental activity by the tegmental part of the fornix, by the mamillary peduncle and by the mamillo-tegmental tract. Cajal (1911) described the mamillo-thalamic fibres arising as collaterals of the mamillo-tegmental tract, a description which suggests an intimate link between the thalamic and tegmental parts of the mamillary system. The present counts of the principal mamillary tract and of the mamillo-thalamic tract support Cajal's description, but the degeneration experiments reported by van Valkenberg (1911-12) indicate that the two efferent mamillary tracts have an independent origin in separate parts of the mamillary region. The present counts have shown that there must be at least as many points of branching as there are mamillo-tegmental fibres, but they have not shown whether the pattern of this branching is that described by Cajal (Text-fig. 5*b*) or that shown in Text-fig. 5*c*. For an understanding of the relationship between the mamillo-thalamic and mamillo-tegmental pathways the work of van Valkenberg must be repeated in conjunction with quantitative observations.



*Quantitative relationships of the mamillary cells and fibres*

In the rabbit and the cat the number of afferent mamillary fibres, the number of mamillary cells and the number of efferent mamillary fibres are all in the region of 100,000. The similarity of these figures is striking but their interpretation in terms of mamillary activity is not possible at present. Analyses of the pattern of branching of the mamillary axons and dendrites and more detailed studies of cell:fibre relationships within the mamillary bodies are essential before mamillary activity can be discussed in detail. The gross quantitative relations are presented here as a basis for further studies of mamillary organization.

The literature dealing with the mamillary connexions suggests that the tegmental connexions of the mamillary bodies are relatively small in primates but larger in the rabbit and the rat. The present counts have shown that there are also certain quantitative differences between the mamillary connexions of the rabbit and the cat. These differences are small and are based on only a few animals, but they suggest that further observations of interspecies differences will prove of interest to a study of mamillary organization. The counts show that the hypothalamic part of the fornix is smaller in the cat than it is in the rabbit, that the mamillary peduncle is larger in the rabbit than it is in the cat and that the lateral mamillary nucleus receives proportionally more coarse mamillary peduncle fibres in the rabbit than the cat. These differences suggest that the hippocampus-mamillary system is not as well established in the rabbit as it is in the cat. In the rabbit more of the hippocampal efferents end in non-mamillary parts of the central nervous system and the mamillary bodies receive proportionally more of their afferent fibres from tegmental sources. The tegmental connexions of the mamillary bodies appear to be particularly small in man (Koelliker, 1896), suggesting the hypothesis that the hippocampus-mamillary system increases at the expense of the hypothalamic and tegmental connexions with increasing cortical development. The fornix counts of the macaque and of man that Simpson (1952) and Daitz (1953) have reported are of particular interest in relation to this problem, but at present it is not possible to relate them to counts of any of the other mamillary connexions in these species. Simpson showed that the post-commissural fornix of the macaque contains about 100,000 fibres, whilst Daitz showed that this bundle contains about 1,000,000 fibres in man. Rose measured the volume of the mamillary nuclei in man (3 months old) and in the macaque (*Macaca mulatta*) and found that the human mamillary bodies are 5 times as large as the monkey's. Although cell counts are not available it appears that the relative size of the fornix is particularly great in man. It would be interesting to know how many of the human fornix fibres pass to the hypothalamus and what proportion of the afferent mamillary fibres come from the tegmentum in man.

The afferent and efferent mamillary fibres link the mamillary bodies, directly or indirectly, with the hippocampus, the septum, the hypothalamus and the midbrain tegmentum. The mamillary bodies are in a position to influence the activity of each of these regions and to be influenced by their activity. Investigations of these parts of the nervous system show that they are concerned with a wide variety of visceral and somatic activities (e.g. Ranson & Magoun, 1939; Hess, 1949; Kaada, 1951; Sprague & Chambers, 1954), but the organization of these activities or their relation

to mamillary activity is not clearly understood. It is probable that further investigations of these regions will make a more detailed analysis of the part that the mamillary bodies play in the activity of the central nervous system possible, but the quantitative interspecies differences that have been described here suggest that this part may not be the same in all mammals.

#### SUMMARY

1. The cells in the mamillary nuclei and the fibres in the mamillary tracts have been counted in the cat and the rabbit.

2. There are about 100,000 cells in the mamillary region; slightly more in the rabbit and slightly fewer in the cat. The lateral mamillary cells form less than 5% of these totals.

3. The number of fibres in the principal mamillary tract is approximately equal to the number of mamillary cells.

4. About 100,000 fibres reach the mamillary bodies in the fornix. The mamillary cells receive less than one-third of their afferent fibres from the mamillary peduncle.

5. The post-commissural fornix loses a large number of fibres between the anterior commissure and the mamillary bodies. This fibre loss is about 60,000 fibres in the cat and 100,000 fibres in the rabbit.

6. The number of fibres in the mamillo-thalamic tract is approximately equal to the number of fibres of the principal tract, showing that a large number of the fibres of the principal tract branch before they enter the mamillo-thalamic and mamillo-tegmental tracts.

7. The counts of the rabbit and the cat material have drawn attention to certain interspecies differences in the relative size of the parts of the mamillary system of connexions. These differences have been discussed in relation to the information that is available for primates.

I wish to thank Prof. J. Z. Young for his advice, criticism and encouragement, and Mr D. A. Sholl for his help with many of the problems that have arisen during the course of this work. I should also like to thank Mr D. Botherel for the photomicrography.

#### REFERENCES

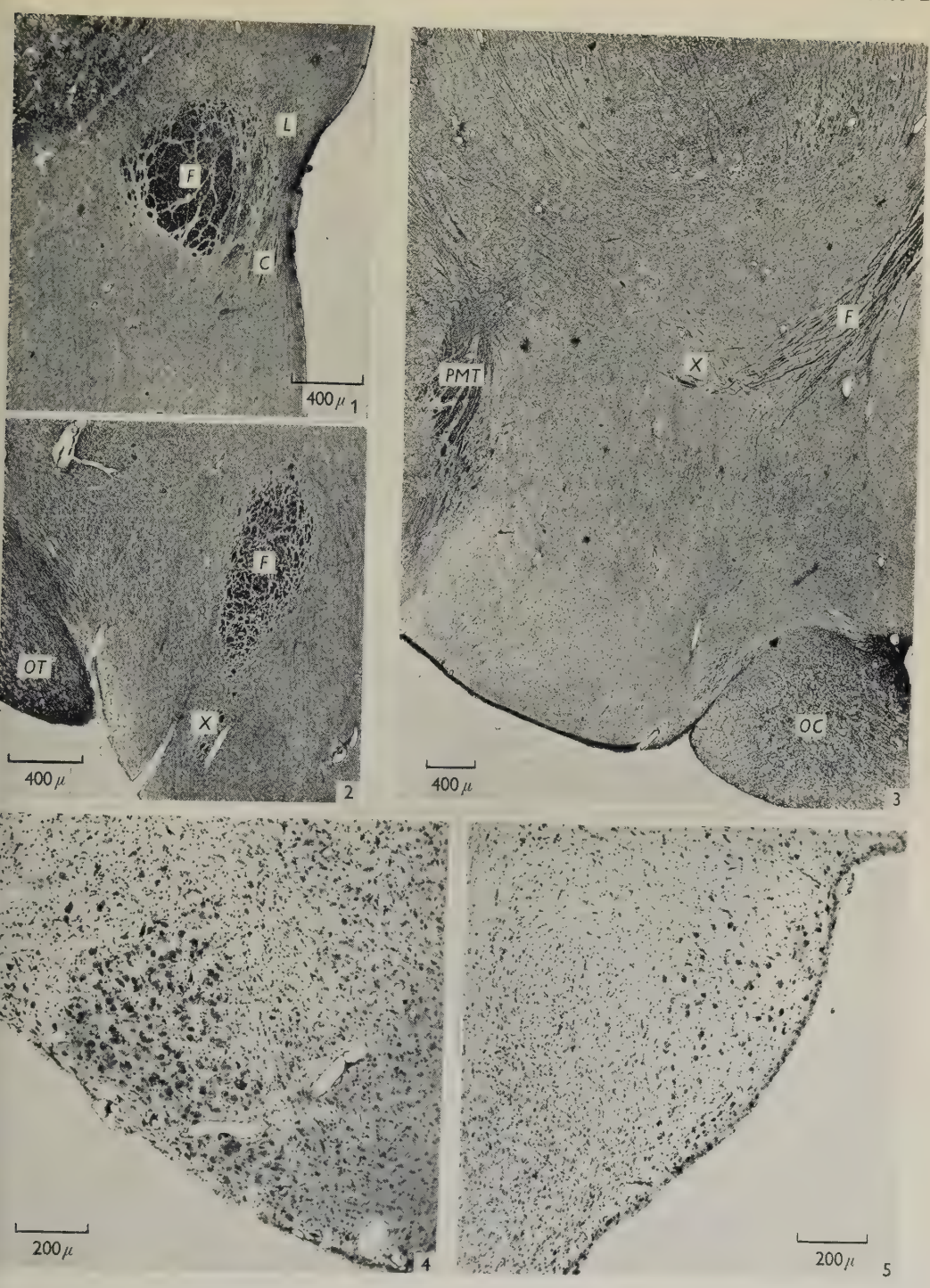
- ABERCROMBIE, M. (1946). Estimation of nuclear population from microtome sections. *Anat. Rec.* **94**, 239-247.
- ADEY, W. R. & MEYER, M. (1952). An experimental study of hippocampal afferent pathways from prefrontal and cingulate areas in the monkey. *J. Anat., Lond.*, **86**, 58-74.
- CAJAL, S. RAMON Y (1911). *Histologie du système nerveux de l'homme et des vertébrés*, 2. Paris: A. Maloine.
- DAITZ, H. (1953). Note on the fibre content of the fornix system in Man. *Brain*, **76**, 509-512.
- EDINGER, L. & WALLENBERG, A. (1902). Untersuchungen ueber den Fornix und das Corpus mamillare. *Arch. Psychiat. Nervenkr.* **35**, 1-21.
- GARDNER, W. D. & FOX, C. A. (1948). Degeneration of the cingulum in the monkey. *Anat. Rec.* **100**, 663-664.
- GEREBTZOFF, M. A. (1941-2). Notes anatomo-expérimentales sur le fornix, la corne d'ammon et leur relation avec diverses structures encéphaliques, notamment éphypysiques. *J. belge Neurol.* **41/42**, 199-206.
- GUDDEN, B. VON (1839). *Gesammelte und hinterlassene Abhandlungen*. Wiesbaden: Bergmann.
- GURDJIAN, E. S. (1927). The diencephalon of the albino rat. *J. comp. Neurol.* **43**, 1-114.

- HESS, W. R. (1949). *Das Zwischenhirn*. Basel: Schwabe.
- HOLMES, W. (1947). The peripheral nerve biopsy. In *Recent Advances in Clinical Pathology*, pp. 402-417. Ed. DYKE, S. C. London: Churchill.
- KAADA, B. R. (1951). Somato-motor, autonomic and electrocorticographic responses to electrical stimulation of 'rhinencephalic' and other structures in primates, cat and dog. *Acta physiol. scand.* **24**, suppl. 83, 1-285.
- KOELLIKER, A. VON (1896). *Handbuch der Gewebelehre des Menschen*, Auf. 6. 2, Leipzig: W. Engleemann.
- KOKEGAMI, H. (1938). Die Kerne und Verbindungsbahnen des Corpus mamillare der Säugetiere. *Z. mikr.-anat. Forsch.* **44**, 181-162.
- KRIEG, W. J. S. (1932). The hypothalamus of the albino rat. *J. comp. Neurol.* **55**, 19-89.
- MORIN, F. (1950). An experimental study of hypothalamic connections in the guinea pig. *J. comp. Neurol.* **92**, 193-213.
- PAPEZ, J. W. (1937). A proposed mechanism of emotion. *Arch. Neurol. Psychiat., Chicago*, **38**, 725-743.
- PAPEZ, J. W. (1938). Thalamic connections in a hemidecorticate dog. *J. comp. Neurol.* **69**, 103-120.
- RANSON, S. W. & MAGOUN, H. W. (1939). The hypothalamus. *Ergebn. Physiol.* **41**, 56-163.
- RIOCH, D. McK. (1929). Studies on the diencephalon of carnivora. Part 1. The nuclear configuration of the thalamus, epithalamus, and hypothalamus of the dog and cat. *J. comp. Neurol.* **49**, 1-119.
- RIOCH, D. McK. (1931). Studies on the diencephalon of carnivora. Part III. Certain myelinated fibre connections of the dog (*Canis familiaris*), Cat (*Felis domestica*), and aevisa (*Crossarchus obscurus*). *J. comp. Neurol.* **53**, 319-388.
- ROSE, J. (1939-40). The cell structure of the mamillary body in mammals and in man. *J. Anat., Lond.*, **74**, 91-115.
- SIMPSON, D. A. (1952). The efferent fibres of the hippocampus in the monkey. *J. Neurol. Psychiat.* **15**, 79-92.
- SPRAGUE, J. M. & CHAMBERS, W. W. (1954). Control of posture by reticular formation and cerebellum in the intact, anaesthetized and unanaesthetized and in the decerebrated cat. *Amer. J. Physiol.* **176**, 52-64.
- SPRAGUE, J. M. & MEYER, M. (1950). An experimental study of the fornix in the rabbit. *J. Anat., Lond.*, **84**, 354-368.
- VALKENBURG, C. T. VAN (1911-12). Caudal connections of the corpus mamillare. *Proc. Acad. Sci. Amst.* **14** (II), 1118-1121.

## EXPLANATION OF PLATE

- Fig. 1. Frontal section through the post-commissural region of the cat. Holmes method. *C*, compact group of fibres passing from the fornix into the periventricular region; *F*, fornix; *L*, loose-fibred group lying dorsomedial to the fornix.
- Fig. 2. Frontal section through the hypothalamus of the cat. Holmes method. *F*, fornix; *OT*, optic tract; *X*, fibres running ventro-laterally from the fornix into the medial forebrain bundle.
- Fig. 3. Parasagittal section through the hypothalamus of the cat. Holmes method. *F*, fornix; *PMT*, principal mamillary tract; *OC*, optic chiasma; *X*, fibres running postero-dorsally from the fornix towards the principal mamillary tract.
- Fig. 4. Frontal section through the lateral mamillary nucleus of the cat. Nissl method.
- Fig. 5. Frontal section through the lateral mamillary nucleus of the rabbit. Nissl method.







# THE ORGANIZATION OF THE VISUAL CORTEX IN THE CAT

BY D. A. SHOLL

*Department of Anatomy, University College, London*

In a previous paper (Sholl, 1953) the nature of the organization of the dendritic fields of cortical neurons was examined. The present study applies quantitative methods to the examination of the axonal distributions of cortical neurons and to the afferent supply to the visual cortex of the cat. The cortex is an aggregate of neurons infiltrated by afferent axons arising from different parts of the nervous system. This aggregate may be divided into subaggregates or groups wherein membership of a particular subaggregate is determined by the distribution of the processes of the neurons. The organization of the cortex is discussed in terms of certain subaggregates and their relationships to the incoming afferent fibres.

Neurons have been classified in accordance with the joint distributions of their axons and dendrites, as revealed in histological preparations made by the Golgi rapid method. These results have been compared with the neuronal density distributions found in Nissl preparations, and both relative and absolute estimates of the densities of the different types of neuron computed. The resulting neuronal organization has been further examined with reference to the afferent supply to the cortex.

These results lead only to a partial understanding of cortical activity but they supply knowledge about the number of afferent fibres, the number of efferent fibres and the quantitative relationships subsisting between these fibre groups and the cortical neurons; this knowledge is essential for progress in cortical physiology.

## METHODS

This study was made on some 500 sections of the visual cortex of 21-day-old kittens stained by the Golgi rapid (osmic-dichromate) method and cut at 100–160  $\mu$ .

Each section was examined for 'completely' stained neurons; this criterion was considered to be satisfied if (*a*) the dendrites of a neuron appeared to be complete and uncut by the knife, and (*b*) if the axon could either be traced into the white matter or, tapering, appeared to end in the cortex. No other neurons were catalogued.

The neurons were classified into seven groups in accordance with the joint distribution of their axons and dendrites; neurons that did not satisfy the criteria of any of the groups were noted separately. This scheme of classification is shown in Fig. 1. Less than 2.5% of the neurons studied failed to satisfy any of the criteria; if the 'inverted pyramid' type of neuron were classified as a deformed stellate cell ( $S_3$ ), then even fewer neurons would be excluded.

Since the thickness of the cortex is highly variable and changes in curvature produce changes in thickness of cortical laminae which do not vary directly with the depth of the laminae, measurements of the absolute and relative depths of cells



are misleading (Bok, 1929). Consequently, the positions of the neurons were described in terms of zones that are easily recognizable in both Nissl and Golgi preparations. These zones are as follows:

- Zone 1 The outermost, almost neuron free, layer.
- Zone 2
- Zone 3 The region of Gennari's line.
- Zone 4
- Zone 5 The region of the deep layer pyramids.
- Zone 6








	Type	Description
	P <sub>1</sub>	Pyramidal cell with unbranched axon to white matter
	P <sub>2</sub>	Pyramidal cell with branched axon to white matter
	P <sub>3</sub>	Pyramidal cell with branched axon to white matter and recurrent collaterals
	P <sub>4</sub>	Pyramidal cell with axon forming recurrent collaterals and branches only
	S <sub>1</sub>	Stellate cell with axon distributed within the dendritic field of the cell
	S <sub>2</sub>	Stellate cell with axon to white matter
	S <sub>2</sub>	Stellate cell with axon to outermost cortical zone

Fig. 1. Diagrams and description of the principal types of neuron found in the cerebral cortex.

Zones, 1, 3 and 5 are clearly recognizable, zones 2 and 4 are merely intercalated between these primary zones, while zone 6 is that part of the cortex between the primary zone 5 and the white matter. In the actual records zones 2 and 6 were subdivided into upper and lower portions but, since these subdivisions appeared to serve no useful purpose, the positions of the various neurons were finally only referred to the six zones described. There is no sharp division between any two zones, with the possible exception of zones 1 and 2.

The distributions of the terminations of the incoming axons were studied on the same preparations.

The sections used for the Nissl preparations were fixed by the perfusion of formol saline, embedded in paraffin and stained by buffered thionine. The total shrinkage factor is of the order 25 %.

## RESULTS

### *The organization of cortical neurons*

The results of this survey are shown in Table 1. Altogether 553 complete neurons were available and thirteen (2.5 %) did not fall within the present classification.

Since the method of staining is selective, it is desirable to have some indication that the types and numbers of cells studied form an adequate representation of the cell populations of the different zones. Complete certainty is unattainable but it is possible to show that the sampling is reasonably representative.

Table 2 shows the results of a comparison between the present sample and Nissl sample. The first two columns show the zones and their approximate thicknesses; column 3 states the cell densities at different depths (Sholl, 1953). Column 4 gives the mean zonal densities and column 5 the total numbers of neurons in the zone,  $k$  being a constant depending on the size of the piece of cortex. Zone 4 is the most sparsely populated zone, and comparison of the totals for the different zones with that of zone 4 will give a measure of the relative numbers of cells in these zones. These relative numbers are shown in column 6. Column 7 shows the total numbers of complete cells for each zone stained by the Golgi method (from Table 1). Again zone 4 has the smallest representation, and comparison with the other zones gives the results shown in the last column.

Comparison of column 6 with the last column shows that, in general, this survey of neurons stained by the Golgi method has produced a proportional representation of the neurons with the possible exception of the lowermost zone. This discrepancy may be due to the difficulty of defining the grey-white boundary in the Nissl preparations and to the possible inclusion of a certain number of neuroglial cells, which are often difficult to distinguish from small neurons in the Nissl picture.

Certain facts are immediately obvious.

(1) The majority of cortical neurons have axons leaving the grey matter and apical dendrites ramifying in the outermost zone of the cortex. These neurons are present at all depths in the cortex with the exception of the outermost layer.

(2) Neurons with their axonal ramifications locally distributed within their own dendritic field are mainly found among the terminations of the afferent fibres arising from the thalamus. There is a smaller concentration of these neurons among the terminations of a second group of afferent fibres.





(3) Neurons whose axons have recurrent collaterals are especially prominent in the lower part of the cortex.

(4) A number of deep-lying pyramidal cells have the terminal ramifications of their apical dendrites in the zone of distribution of the terminations of fibres from the thalamus.

For some purposes it is convenient to express the results of the counts in terms of the actual numbers of cells per unit volume of cortex rather than merely in terms of the sample numbers. This is done by first expressing the numbers of each type of cell as a fraction of the total number in the zone and then transforming this ratio to the number of cells/ $10^6 \mu^3$  of cortex by means of the known cell densities found from Nissl preparations. For example, the number of pyramidal cells with unbranched axons to the white matter ( $P_1$ ) in zone 2 found in the sample is 53 and the total number of cells sampled in the zone is 189 (Table 1). The Nissl density is  $82/10^6 \mu^3$  and the estimated number of cells of this type in each  $10^6 \mu^3$  of zone 2 is  $(53 \times 82)/189 = 23$ .

The distribution of the types of neuron at different cortical levels and the manner in which the distribution of a given type varies with depth could be shown by means of a set of histograms. These different distributions are more easily visualized by means of the contour diagram shown in Fig. 2.

In this diagram the different types of neuron are shown along the top and the cortical zones, with their relative thicknesses, on the left-hand side. Vertical lines show that the neurons have been classified discretely and the absence of horizontal lines emphasizes the lack of sharp boundaries between the various zones. The numbers denote the estimated number of neurons of a given type contained in a cortical volume of  $10^6 \mu^3$  to be found within the appropriate zone. The contour lines are drawn at 5-neuron intervals in the same way as contours for changes in height on a map. The different varieties of distribution may be found by drawing either horizontal or vertical lines across the diagram. Horizontal lines will give the distribution of the different neuronal types at a given depth, whereas vertical lines will give the distribution of a given neuronal type with changing depth.

The dendritic distributions of cortical neurons have been studied previously (Sholl, 1953). As a result of further measurements it may be said that the extent of the basal dendrites appears to be normally distributed about a mean radius of  $160 \mu$  with a standard deviation of  $45 \mu$ : the standard deviation of the mean is approximately  $10 \mu$ .

### *The afferent fibres to the visual cortex*

There appear to be three groups of fibres bringing impulses to the visual cortex:

(1) Those whose terminations are concentrated around the region of Gennari's line (zone 3).

(2) Those with terminations mainly between Gennari's line and the outermost layer of the cortex (zone 2).

(3) The tangentially running fibres in the outermost zone (zone 1).

The first group, whose extensive terminal branches were noted by Cajal and very well illustrated by O'Leary (1941), enter the cortex as comparatively coarse fibres and appear to arise mainly from the lateral geniculate body. The present

study confirms the distributions of the terminations of the fibres described in this previous work and measurements have been made on the extent to which the terminations of any one geniculate fibre may ramify. From measurements on a small sample the mean distance between the tips of the most widely spread branches was found to be  $650\mu$  and it must be emphasized that a single thalamic fibre may have a termination in the lower part of zone 3 and another in the upper part of this zone, the tips being also widely separated in a direction parallel to the pial surface.

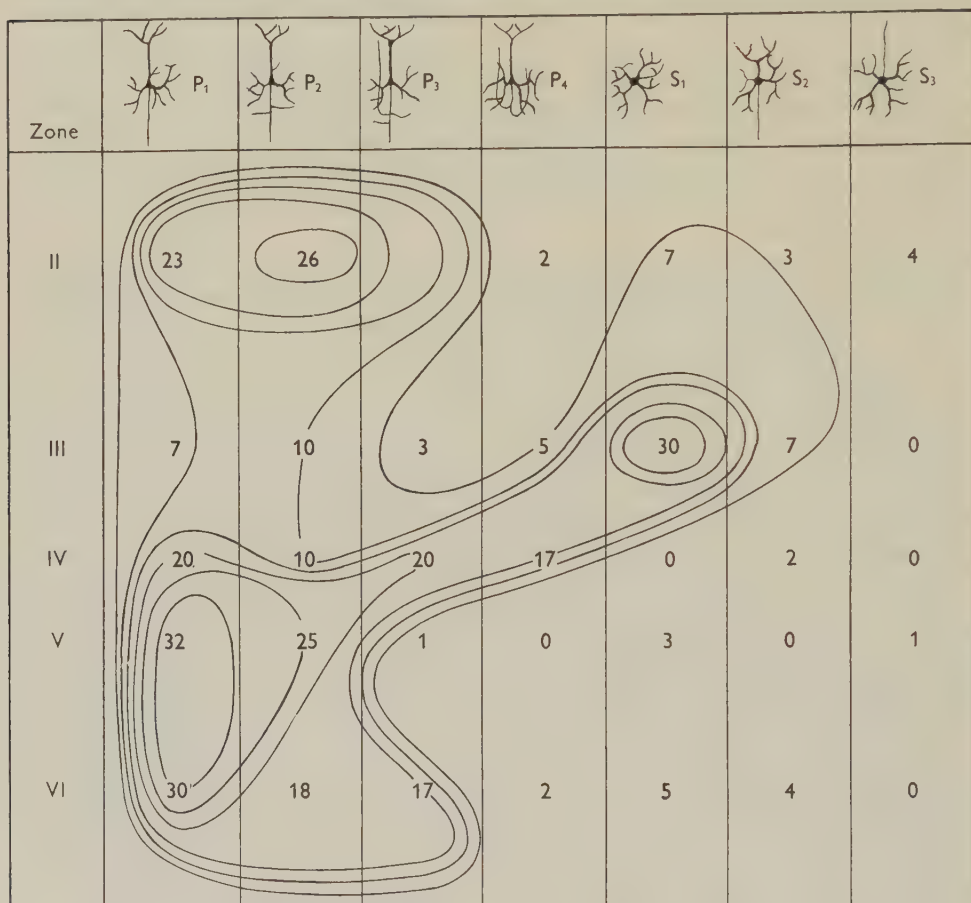


Fig. 2. Contour diagram showing the numbers of cells of different types contained in a cortical volume of  $10^6\mu^3$  in the different cortical zones. Contour lines are drawn for densities of 5, 10, 15, 20, 25 and 30 neurons per  $10^6\mu^3$  of cortex.

The axons of the second set of incoming fibres are much thinner and again these branch before terminating. They are said to be commissural and association fibres (Lorente de Nó, 1949; Chang, 1953; Nauta, 1954), but their origin is still uncertain.

The set of very fine axons running in the outermost layer of the cortex is the most difficult of all to study. Staining is difficult with all the techniques that have been tried, but good silver impregnations (Bielschowsky and Holmes) show that these

fibres are present in large numbers. The only positive evidence of their origin found in the present work is that a number of deeper lying cortical neurons have axons running to this layer and then turning to run tangentially.

An estimate of the density of afferent fibres leaving the white matter was made by making a number of counts of the total number of fibres as stained in silver preparations cutting a unit area of the grey-white boundary in silver preparations of sections of measured thickness cut perpendicularly to the pial surface. This

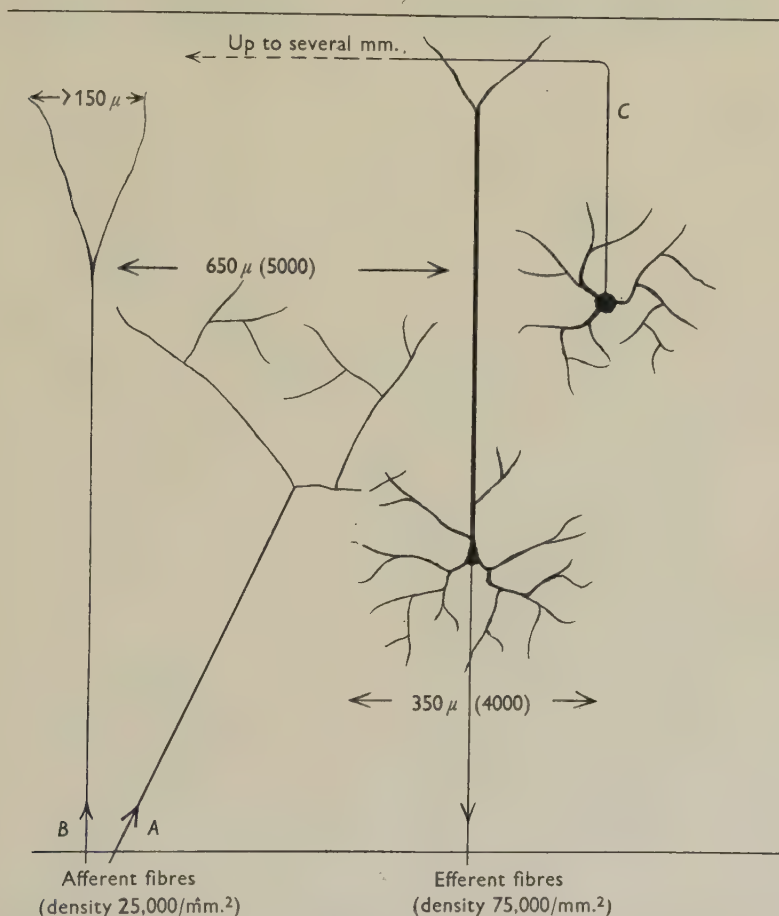


Fig. 3. Diagram to illustrate certain quantitative features in the visual cortex of the cat. Numbers in brackets denote the approximate numbers of neurons under the immediate influence of two of these systems.

was found to be of the order  $10^5$  fibres per mm.<sup>2</sup>. Since 70% of all the cortical neurons have axons running into the white matter, the number of fibres leaving the cortex can be estimated from the neuronal densities found in Nissl preparations. The estimated density of such axons is of the order  $75 \times 10^3$  axons per mm.<sup>2</sup>.

Some of the quantitative relationships subsisting between the afferent fibres are shown diagrammatically in Fig. 3.



The non-recurrent collaterals usually leave the main axon within the basal dendritic field of their parent neuron, i.e. within  $200\mu$  of the cell body, and many of them do not extend outside the zone of the basal dendrites. However, it is not unusual to find branches stretching for more than 1 mm. The recurrent collaterals of neurons in the upper part of the cortex usually have their terminations within the basal dendritic field of their cell of origin but in the deeper parts of the cortex

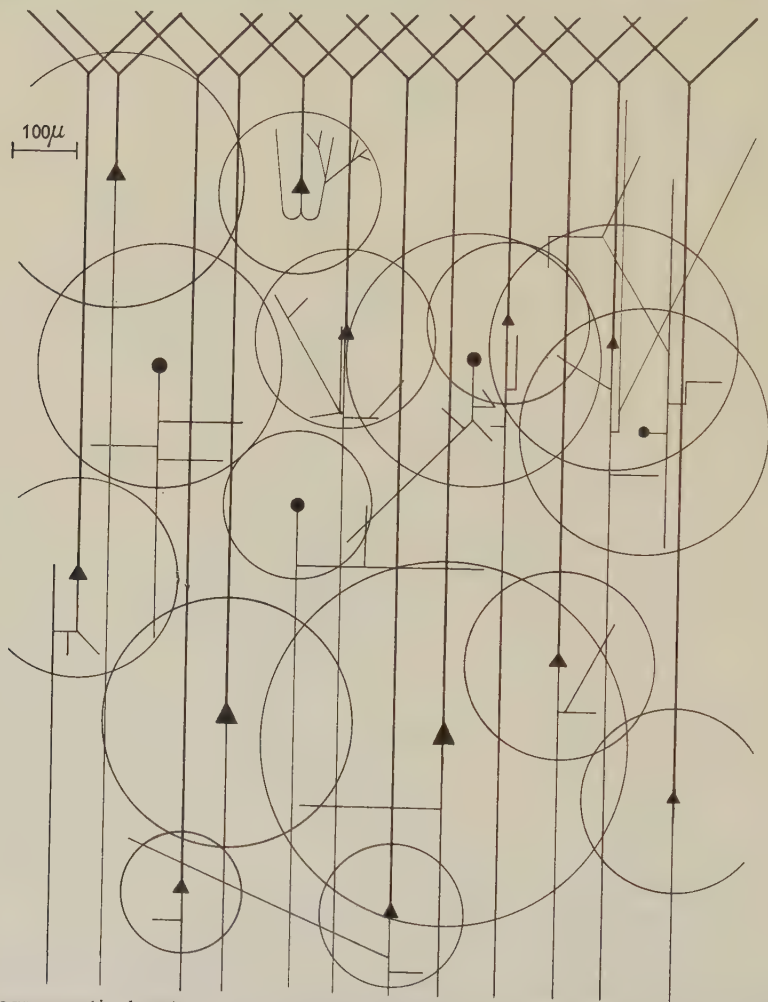


Fig. 4. Diagrammatic drawing of a number of cortical neurons with their axons, dendritic fields and axonal branches drawn to scale.

the recurrent collaterals extend much further but none has been found with terminations above the Gennari region. Some of these facts are illustrated in Fig. 4.

Many of the implications of these quantitative results are most easily seen from a diagram constructed in the following way. The Nissl studies (Table 2) show that, excluding the almost neuron-free outermost zone, the ratios of the numbers of cells in the different zones are approximately 5 : 3 : 1 : 2 : 4, and the Golgi studies show

that in the zone with the fewest cells (zone 4), four types of neuron must be represented even if types that form less than 5 % of the total are ignored. Consequently, in order to preserve the proportional relationships in their simplest form we must consider a column of cells with four neurons in zone 4 and hence twenty cells in zone 2, twelve in zone 3, eight in zone 5 and sixteen in zone 6. Furthermore, the total number of neurons represented in each zone must be subdivided in proportion

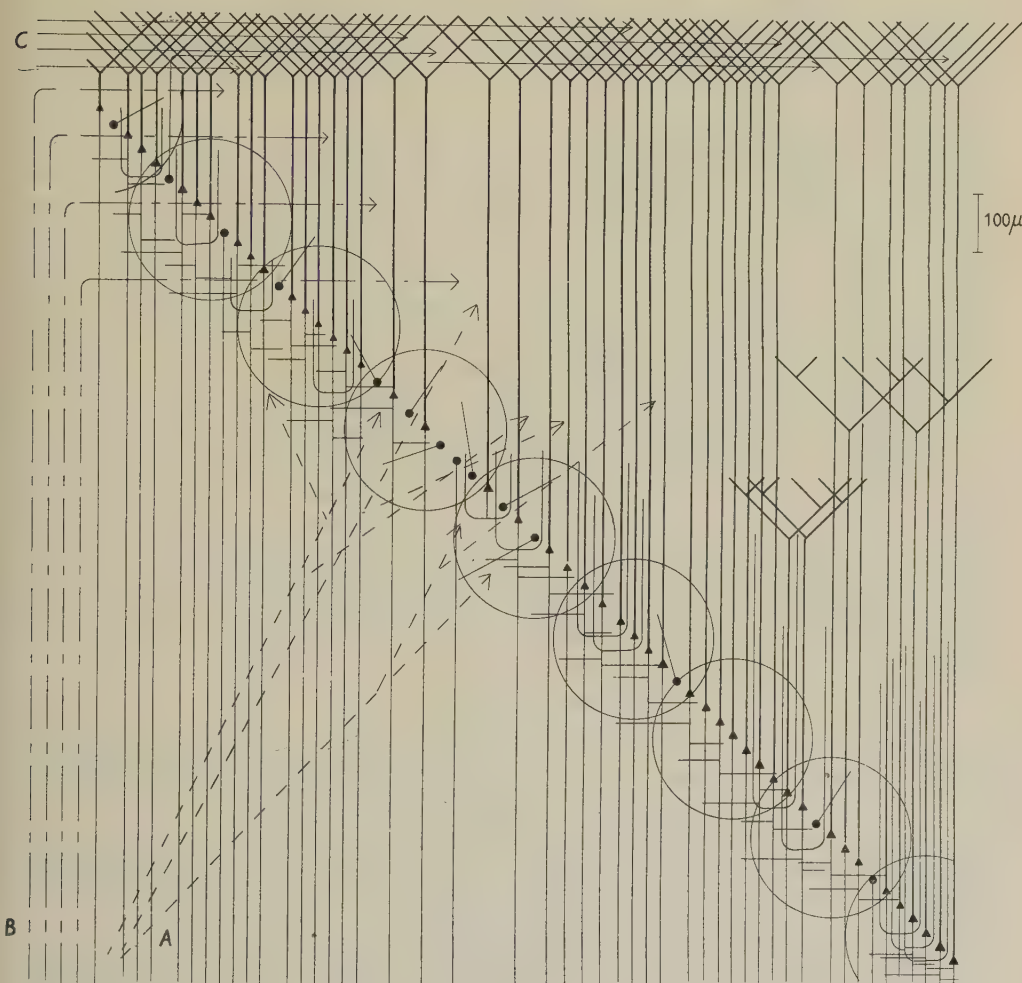


Fig. 5. Diagram to show the distribution of the different types of neuron in the visual cortex of the cat. The three sets of afferent fibres (A, B, C) and their terminations are also shown.

to the number of each type found in that zone. The diagram shown in Fig. 5 has been constructed on this principle. The representative column of cells has been staggered in order to make the separate axonal ramifications clear; it must be remembered that each neuron in the figure represents a group of cortical neurons. The destinations and directions of the axons and their branches are preserved but individual variations are not shown (see Fig. 4). The manner of termination of the

apical dendrites has been formalized, and the extent of the basal dendritic fields shown by circles each with a radius equal to that of the mean dendritic field. The general distributions of the three sets of afferent fibres (A, B and C) are shown formally. The diagram attempts to illustrate a statistical average of cortical organization.

#### DISCUSSION

The cerebral cortex is an organization of neurons and their processes such that streams of impulses arriving from various sources along afferent fibres interact with one another and with some engram derived from the past history of the animal. The impulses resulting from this activity lead to changes in behaviour and in the cortical engram. Any attempt to discuss the mode of activity of the cortex demands hypotheses relating to the properties of neurons and the manner in which impulses propagated along an axon may affect other neurons. The precise nature and method of operation of cortical synapses is unknown, but it seems reasonable to assume that activity associated with axons that are sufficiently near to the perikaryon and dendrites of a neuron will lead to a local depolarization of its surfaces. If this depolarization is sufficiently extensive as a result of spatial and temporal summation, an action potential will be propagated along the axon and its branches; moreover, the production of such an action potential leads to some shorter or longer change in the condition of the perikaryon and dendrites.

The present work is an attempt to make a quantitative study of some features of the cortex by considering the relative sizes and relationships of subaggregates or groups of neurons, membership of a group being determined by certain geometrical properties of the axons and dendrites. Size must be a parameter of first importance in determining the part played by each neuron. For example, the wider is the dendritic field of a cell, the greater the number and variety of influences falling upon it.

#### *The cortical zones*

The zones used in the present study were defined in order to describe the positions of the cell bodies in an objective way that avoids reference to the highly variable thickness of the cortex. They are equivalent to zones marked out in commencing an ecological survey of a district; once the map has been made these artificial boundaries are discarded and the terrain is examined as a whole. In another region of the cortex different zones would almost certainly be convenient, for Gennari's line would no longer serve as a landmark.

The relationship between these zones and the various schemes for cortical lamination will not be described since no importance, tectogenic or otherwise, is ascribed to the zones.

#### *The afferent fibres to the visual cortex*

Many fibres can be traced from the white matter to their terminations. The origin of these fibres can only be rigorously determined by degeneration methods. The fibres that end in the region of Gennari's line (group A) have been studied extensively by Poliak (1927), who concluded that they had their origin in the lateral geniculate body. The nature of the branching of these fibres in the cat has been



described and illustrated by O'Leary (1941) who says that: 'The horizontal branches of the exogenous fibres within the stria are significantly longer than is generally believed, and may issue as many as twelve secondary branches each of which ramifies more extensively.' The present work confirms this statement; the final terminal twigs may be separated by distances of the order of 1 mm. It follows from the estimates of cell density (Sholl, 1953) that up to 5000 neurons and a volume of 0.1 mm.<sup>3</sup> of cortex are within the immediate zone of influence of impulses transmitted by a single thalamic fibre. The more precise description of the mode of branching and of the extent of the terminal ramifications of these fibres requires further examination.

The second set of incoming fibres (B fibres) appears to have its main terminations superficial to the Gennari zone. These fibres are considerably thinner on emergence from the white matter than the group A fibres, and their source is still speculative. The third set (C fibres) which forms the fine tangentially running fibres of the outermost layer of the cortex and is immersed in the dense ramifications of apical dendrites also has an unknown origin. Undoubtedly a number of the fibres are the axons of deeper lying cortical neurons, but whether these axons form the major part of this set cannot be stated at present. Both the origin and extent of the ramification of these axons are under investigation. Nauta (1954), working with his degeneration method, has described intra-cortical and callosal fibres ending in all layers of the cortex. Details of this work are not yet available and further correlated studies of degeneration and Golgi preparations must be made.

#### *The efferent fibres from the cortex*

The present work emphasizes that axons running into the white matter originate from all levels of the cortex with the exception of the outermost layer and, in fact, the majority of the cortical neurons have axons of this kind. Little can be said about the destination of these axons; Dusser de Barenne (1934) and Le Gros Clark & Sunderland (1939) provide evidence that many of the axons arising from the deep lying neurons travel to subcortical structures. The destination of the axons from the upper layers of the cortex is uncertain.

#### *The general organization of the visual cortex*

The diagram shown in Fig. 5 represents not only the manner in which the cortical neurons are related to the different sets of afferent fibres but also indicates the relationship between the neurons themselves. For simplicity only a single column of cells has been shown, and even here a single neuron in the diagram represents a group of actual neurons. Furthermore, it must not be assumed that such a column of cells indicates the existence of some kind of cortical unit; the branches of each afferent fibre come into relationship with the neurons of a number of such columns and the axons of any column have branches ramifying to other columns. The variation in the extent of inter-neuronal interaction is shown more clearly in Fig. 6, where a number of neurons have been drawn to scale to show the varied manner of axonal branching and the overlap of dendritic fields.

Bearing in mind the hypotheses stated earlier we may consider the situation shown in Fig. 5 and in a more simplified and less exact form in Fig. 6. Here the main

principles of the more complicated figure are preserved but it is no longer possible to show the different types of neurons in their correct proportions when the total number of neurons depicted has been so radically reduced. Examination of Figs. 2 and 5 makes the manner of distribution of the principal subaggregates or groups clear. A large group of stellate cells is associated with the terminations of the A sets

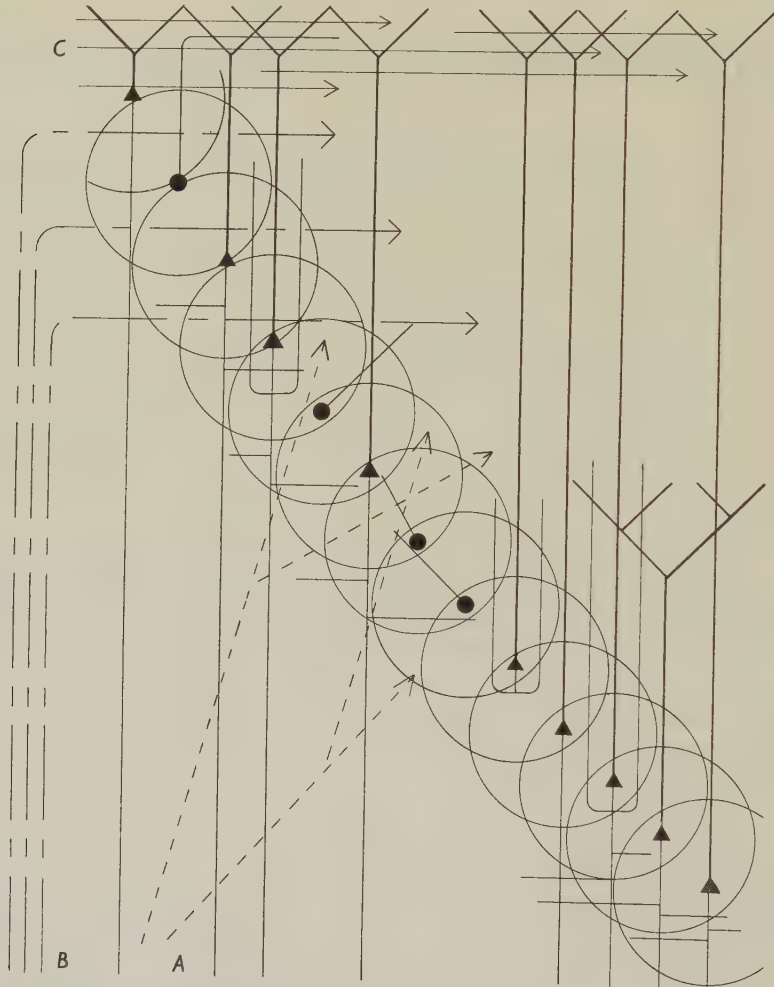


Fig. 6. A simplified diagram of the relationships between cortical neurons and the afferent fibres to the cortex. The main principles illustrated in Fig. 5 are preserved but the proportions of the different types of neuron are less accurate.

of afferent fibres and a smaller group with the B set. Large groups of pyramidal cells, showing branched and unbranched axons in approximately equal numbers, are found in the upper and lower layers of the cortex while neurons with recurrent collaterals are mainly concentrated below the Gennari zone. Neurons with axons running into the outermost zone occur mainly in the upper part of the cortex.

Other much smaller subaggregates occur but it is suggested that they play so small a part in the general cortical organization that they may be ignored.

If these diagrams are now examined more closely from a purely static point of view, and remembering that each neuron in the drawings represents a group of cortical neurons, it is immediately apparent that impulses arriving along a single thalamic fibre will be dispersed amongst the 5000 neurons distributed around its terminal branches. The precise number of neurons within this set influenced by the afferents is unknown, but it may be that as much as 0.1 mm.<sup>3</sup> of cortex has its state modified. Activity may spread from the neurons nearest to the terminal branches and may lead not only to impulses leaving the cortex but to activity in further groups of neurons above and below this region. The presence of a large group of neurons with recurrent collaterals beneath the Gennari zone suggests that the effects of the downward flow of activity will lead to secondary influences at the higher level. This type of activity might be of the 'reverberating circuit' type suggested by Lorente de Nó (1933, 1934).

A similar examination of the afferent fibres of set B again shows that some of the neurons under the immediate influence of these fibres give rise to impulses leaving the cortex, while others also mediate impulses that travel back to the region of the distribution of the afferent terminations. In this case, however, a greater proportion of the neurons directly associated with the afferent terminations have axons that leave the cortex; in both cases, recurrent collaterals convey impulses back to the primary afferent region. Impulses travelling along the tangential axons of the outermost part of the cortex will influence some 70 % of all cortical neurons through the ramifications of the apical dendrites.

The cortex is a dynamic system and important as these purely spatial and static considerations may be, they would be most misleading if considered apart from the temporal relations existing between the activities of the neurons and the different sets of afferent fibres. The method adopted in this study gives no information about the connectivity of individual neurons with one another and with the afferent fibres and, consequently, discussion of the temporal factors involved would be purely speculative. Further important parameters may emerge from similar investigations on different areas of cortex and from phylogenetic and ontogenetic studies.

This work shows that some aspects of the problem of cortical organization may be approached from a statistical point of view in the sense that the parameters of groups of elements are considered. The value of this method has been clearly shown in the development of statistical physics in which the states of an aggregate composed of a large number of elements have been investigated. In the systems so far studied by statistical mechanics certain assumptions regarding the homogeneity of the elements are acceptable; no such homogeneity can be assumed for the neurons of the cerebral cortex. Adequate statistical methods for resolving these problems have still to be found.



## SUMMARY

1. A quantitative analysis of the distribution of the neurons of the visual cortex of the cat has been carried out in accordance with the joint distributions of their axons and dendrites.

2. The study of Golgi and Nissl preparations enables both the relative and absolute densities of the different types of neuron to be estimated.

3. The majority of cortical neurons have axons leaving the grey matter and apical dendrites ramifying in the outermost zone of the cortex.

4. Neurons with their axonal ramifications locally distributed within their own dendritic field are mainly found among the terminations of two sets of afferent fibres.

5. Neurons whose axons have recurrent collaterals are especially numerous in the deeper part of the cortex.

6. A number of deep lying pyramidal cells have the terminal ramifications of their apical dendrites in the zone of distribution of the terminations of fibres from the thalamus.

7. Three sets of afferent fibres are described.

8. Fibres arising in the lateral geniculate body branch extensively within the cortex, the distance between the tips of their terminal branches being of the order of 0.5 mm. Each thalamic fibre may directly influence the state of about 0.1 mm.<sup>3</sup> of cortex in which lie 5000 neurons.

9. The density of efferent fibres from the cat visual cortex at the grey-white boundary is about 75,000 fibres/mm.<sup>2</sup>. Approximately 25,000 fibres/mm<sup>2</sup> enter the cortex from the white matter.

Once again I am most grateful to Prof. J. Z. Young for his unstinted help and criticism. Dr R. Lorente de Nó very kindly gave me one of the series of slides used in this study. I must also thank Mr P. H. Wedd for his assistance throughout this work.

## REFERENCES

- BOK, S. T. (1929). Der Einfluss der in den Furchen und Windungen auftretenden Krümmungen der Grosshirnrinde auf die Rindenarchitektur. *Z. ges. Neurol. Psychiat.* **121**, 682-750.
- CHANG, H. T. (1953). Cortical response to activity of callosal fibres. *J. Neurophysiol.* **16**, 117-132.
- DUSSER DE BARENNE, J. G. (1934). Origin of motor reactions produced by electrical stimulation of the cerebral cortex. *Arch. Neurol. Psychiat., Chicago*, **31**, 1129-1137.
- LE GROS CLARK, W. E. & SUNDERLAND, S. (1939). Structural changes in the isolated visual cortex. *J. Anat., Lond.*, **73**, 563-574.
- LORENTE DE NÓ, R. (1933). Studies on the structure of the cerebral cortex. I. The area entorhinalis. *J. Psychol. Neurol., Lpz.*, **45**, 318-348.
- LORENTE DE NÓ, R. (1934). Studies on the structure of the cerebral cortex. II. Continuation of the study of the ammonic system. *J. Psychol. Neurol., Lpz.*, **46**, 113-177.
- LORENTE DE NÓ, R. (1949). Cerebral cortex. In Fulton's *Physiology of the Nervous System*, pp. 288-315. Oxford.
- NAUTA, J. H. (1954). Terminal distributions of some afferent fiber systems in the cerebral cortex. *Anat. Rec.* **118**, 333.
- O'LEARY, J. L. (1941). Structure of the area striata of the cat. *J. comp. Neurol.* **75**, 131-164.
- POLIAK, S. (1927). An experimental study of the association, callosal and projection fibres of the cerebral cortex of the cat. *J. comp. Neurol.* **44**, 197-258.
- SHOLL, D. A. (1953). Dendritic organization in the neurons of the visual and motor cortices of the cat. *J. Anat., Lond.*, **87**, 387-406.

# FURTHER OBSERVATIONS ON THE BEHAVIOUR OF NUCLEAR STRUCTURES DURING DEPLETION AND RESTORATION OF NISSL MATERIAL

BY HUGH A. LINDSAY AND MURRAY L. BARR

*Department of Microscopic Anatomy, University of Western Ontario,  
London, Canada*

## INTRODUCTION

The work to be reported is an extension of observations which were recorded previously by Barr & Bertram (1951) in this *Journal*. In the earlier study, depletion and restoration of the Nissl material in hypoglossal neurons of the cat were found to be accompanied by a reversible swelling and vacuolation of the nucleolus. This observation supported the view that the nucleolus participates in some way in the synthesis of Nissl material. Concurrently, the sex chromatin (formerly called the nucleolar satellite) moved temporarily from its usual juxtannucleolar position toward the nuclear membrane. The observations of Barr & Bertram were made on sections stained with cresyl violet. It was considered advisable to repeat the experiments, using the methyl green-pyronin and Feulgen staining methods, which are capable of giving cytochemical information with respect to the nucleic acids.

Brusa (1952) suggested that the sex chromatin is either the basophile clot of Levi or the accessory body of Cajal. These nuclear structures have been well known to neurocytologists for many years (Ramón y Cajal, 1910). The methyl green-pyronin and Feulgen stains are suitable for investigating any possible relationship between the sex chromatin and the basophile clot of Levi. In order to study the relationship between the sex chromatin and the accessory body of Cajal, an argyrophile structure, sections from each animal were stained with protargol. Sections stained by silver methods are of additional interest, since they show details of nucleolar structure which are not visible in material stained by other methods.

A brief introductory comment on the sex chromatin may be helpful, since the probable significance of this intranuclear structure has only recently been understood. The current conception of the sex chromatin is described in more detail by Moore & Barr (1953) in connexion with a comparative study of this structure in nerve cells of several mammals. In various species of the orders Carnivora and Artiodactyla, the sex chromatin is clearly visible in cells of females, where it is of the order of  $1\mu$  in diameter. A distinct mass of sex chromatin is seldom visible in nerve cells of males in these species. The position of the sex chromatin within the nucleus varies with the type of neuron and from one species to another. Sex chromatin has not been identified in nerve cells of animals belonging to the orders Rodentia and Lagomorpha. In cells other than neurons, the sex chromatin of females almost always takes the form of planoconvex body lying against the nuclear membrane (Graham & Barr, 1952; Moore & Barr, 1954). A sex difference in the morphology of intermitotic nuclei is present in cat embryos, even in early stages

before gonadal differentiation has taken place (Graham, 1954*a, b*). Such evidence as is available suggests that the sex chromatin of female cells is formed by a fusion of heterochromatic portions of the two *X*-chromosomes, while the *XY*-chromosomes of males do not form a structure of comparable size. The observations which have been summarized are the basis of the skin biopsy test of chromosomal sex in hermaphrodites (Moore, Graham & Barr, 1953; Barr, 1954).

#### MATERIALS AND METHODS

Seventeen mature cats (fifteen females and two males) were used, the predominance of females being a consequence of the inconspicuous nature of the sex chromatin in males. Chromatolysis was produced in cells of the right hypoglossal nucleus by electrical stimulation of the hypoglossal nerve; cells of the left hypoglossal nucleus served as a control. The nerve was stimulated with bipolar platinum electrodes at a frequency of 50 cye./sec., with a pulse duration of 1 msec., using a stimulator with a square wave output. The voltage was increased gradually from 10 to 20 V. during the first hour and maintained at 20 V. during the succeeding 7 hr. Anaesthesia was maintained throughout the period of stimulation by intraperitoneal administration of Nembutal. The procedure of nerve stimulation was used empirically, since this method was shown in the earlier experiments to cause chromatolytic changes of moderate severity and relatively short duration. The simpler procedure of crushing the hypoglossal nerve would have served the purpose equally well (Crouch & Barr, 1954). No inferences are being drawn at this time concerning the cause of the chromatolysis which follows axon stimulation under the conditions of these experiments.

Survival times varied between 1 and 18 days, when the animals were sacrificed by perfusion with isotonic 10% formalin by way of the aorta. Paraffin sections, 10  $\mu$  in thickness, were stained as follows. The methyl green-pyronin staining procedure of Kurnick (1952*a*) was followed. Methyl green is selective for desoxyribose nucleic acid (DNA) (Kurnick, 1950). Pyronin may be considered as selective for ribose nucleic (RNA) when applied to the neuron, although such specificity is not general (Taft, 1951). The Feulgen procedure, as outlined by Stowell (1945), was used. A positive Feulgen reaction is specific for DNA when the procedure is followed carefully. Examination of the cells, which contain little cytologically demonstrable DNA, was facilitated by the use of an orange-G counterstain. The protargol staining procedure described by Conn & Darrow (1943) was followed in detail. Finally, the right hypoglossal nerves were stained with osmic acid. It may be stated here that the nerves appeared normal for survival periods of 1 and 2 days; there was mild to moderate Wallerian degeneration from 3 to 7 days; and advanced Wallerian degeneration was present from 9 to 18 days. The stimulating current had obviously caused structural damage to the axis cylinders in each animal.

The alterations in the cells of the right hypoglossal nucleus (stimulated side) were assessed against the appearance of the cells in the left hypoglossal nucleus (unstimulated, control side), using sections stained with methyl green-pyronin. A rough measurement of the depletion and restoration of Nissl material was obtained by a modification of the method of Campbell & Novick (1946), which involves classifying the cells according to the degree of chromatolysis. No particular difficulty was



experienced in classifying the cells during dissolution of the Nissl material. Classification of cells was difficult during the recovery period since typical, discrete Nissl bodies were not reformed throughout the cytoplasm until some time after the amount of Nissl material, judging from the intensity of staining, had returned to normal. Although this procedure cannot be expected to give precise information concerning the depletion and restoration of Nissl material, it seems to be the best method of evaluation available at this time, where a large number of cells is to be considered.

Changes in the size of the nucleolus during chromatolysis were also followed in methyl green-pyronin preparations. Two dimensions (at right angles to each other) of fifty nucleoli in each hypoglossal nucleus of each animal, were measured with a filar micrometer eyepiece. The profile area of each nucleolus was obtained from the formula  $\frac{1}{4}\pi ab$ , where  $a$  and  $b$  are the diameters.

In sections stained with methyl green-pyronin, the position of the sex chromatin was recorded as adjacent to the nucleolus, free in the nucleoplasm or adjacent to the nuclear membrane, in 500 cells of control and experimental hypoglossal nuclei in each animal. The topography of the accessory body of Cajal was established in a similar manner, using protargol preparations.

#### OBSERVATIONS

Depletion of the *Nissl material* begins during the first day after stimulation and is progressive during the second and third days (Pl. 1, figs. 1, 2). Compact masses of Nissl material, closely applied to the nuclear membrane, are a prominent feature during the latter part of this period. These nuclear caps vary from small, more or less triangular masses to large crescentic accumulations of Nissl substance. There may be more than one to a cell and they may surround almost the whole extent of the nuclear membrane as seen in section. There is an intimate relationship between the nuclear caps and the nuclear membrane, and the nucleus is sometimes indented at the site of a nuclear cap (Pl. 1, fig. 2). The recovery period begins between the second and third day, the rate of restoration of the Nissl material varying considerably from cell to cell. In general, the newly formed Nissl material is more abundant in the perinuclear zone than at the periphery of the cell. Discrete nuclear caps are not often seen during the recovery phase. Many cells are hyperchromatic by the seventh day. The hyperchromasia persists for several days and gradually subsides by the fifteenth or sixteenth day. Shortly thereafter, most of the cells have a normal appearance with respect to both the amount and the distribution of the Nissl material. The general appearance during the recovery phase is one of restoration of Nissl material from the nucleus toward the periphery.

The *nucleolus*, which has been shown repeatedly to contain RNA, is Feulgen-negative (Pl. 1, figs. 3, 4) and stains with pyronin. Very small Feulgen-positive (DNA) particles, which also stain with methyl green, adhere to the nucleolar surface. Their size, distribution and frequency are similar in normal and experimentally altered cells. There should be no confusion between these minute perinucleolar particles of chromatin and the sex chromatin of female cells, in the majority of nuclei in good technical preparations. Perinucleolar chromatin which could be interpreted as basophile clots of Levi is, in our experience, seldom encountered in

hypoglossal neurons of the cat. Reasons for not considering the female sex chromatin as a basophile clot of Levi will be presented in the discussion.

A significant increase in the size of the nucleolus occurs during the third day after stimulation. The enlargement continues during the fourth and fifth days, with a maximum enlargement (mean values) of nearly 30 % in profile area or 50 % in volume. Thereafter, the mean nucleolar size decreases in an irregular manner from one animal to another. These results confirm the observations of Barr & Bertram (1951) which were made on nucleoli stained with cresyl violet.

There is an interval of a little over one day between the first indication of chromatolysis and the first significant increase in the size of the nucleolus. The main enlargement of the nucleolus occurs during the period of restoration of the Nissl material. These observations, together with the appearance of nuclear caps in early stages and the general tendency toward restoration of the Nissl material from the nuclear membrane outward, point to nucleolar participation in the synthesis of Nissl material.

Approximately spherical areas of varying size, staining less intensely than the remainder of the nucleolus with pyronin, are referred to as vacuoles. Vacuolation varies under normal conditions with the type of neuron and the species. Nucleolar vacuolation is encountered in 10 %, or less, of normal hypoglossal neurons in the cat, and then there is usually but one small vacuole. The vacuoles increase in number and size during depletion and restoration of the Nissl material. Occasionally a large vacuole in experimentally altered cells is seen to protrude from the periphery of the nucleolus. There is a slight increase in the incidence of vacuolated nucleoli 2 days after stimulation and their frequency increases to about 80 % during the next 7 days. Vacuolation subsides thereafter until the normal appearance of the nucleoli is restored by the eighteenth day. There is a close association between nucleolar vacuolation and enlargement of the nucleolus, suggesting that the formation of vacuoles may contribute to nucleolar swelling.

Nucleoli stained with protargol show a large number of argyrophile spherules throughout their interior in both control and experimentally altered cells (Pl. 1, figs. 5, 6). Vacuoles are rarely visible in nucleoli of control cells stained with protargol. Nucleolar vacuolation is seen in chromatolytic neurons, although this is not very striking unless the stain is quite light.

There is no apparent change in the position of the nucleolus during dissolution and recovery of the Nissl material. Double nucleoli are encountered in both control and experimental hypoglossal nuclei, but with extreme rarity.

The *sex chromatin* of females is an approximately spherical body about  $1\mu$  in diameter, and is seen in about 80 % of cells which contain the nucleolus in the plane of the section. It is only with great rarity that two structures with the size and staining characteristics of the sex chromatin are encountered in the same nucleus. The sex chromatin in both normal and chromatolytic cells is Feulgen-positive (Pl. 1, figs. 3, 4) and has a strong affinity for methyl green, showing that its main nucleic acid component is DNA. Although the staining reaction of the sex chromatin is not changed in chromatolytic neurons, there are important changes in the position and, to a lesser degree, in the size of the sex chromatin.

There is some variation in the topography of the sex chromatin on the control

side from one animal to another. The average values are as follows: adjacent to the nucleolus 61 %, free in the nucleoplasm 15 %, and adjacent to the nuclear membrane 6 % (total 82 %). It should be emphasized that the topography of the sex chromatin, as noted above, applies specifically to the hypoglossal nucleus of the cat, and that this varies with the type of neuron and the species. In nerve cells of the monkey, for example, the sex chromatin is more likely to be found against the nuclear membrane (Prince, 1952), a position characteristic of cells other than neurons in the cat and other species.

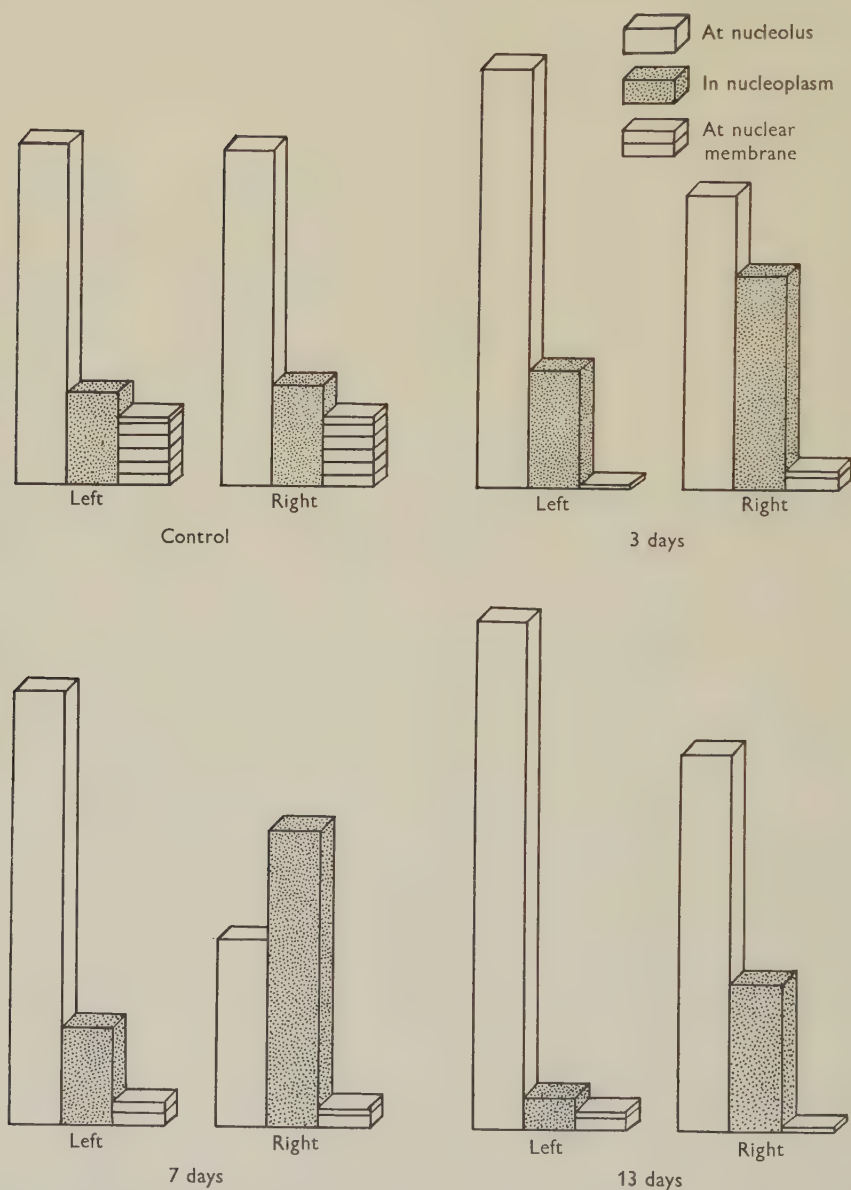
In spite of the variation in position of the sex chromatin in normal hypoglossal neurons of different animals, there is no difficulty in recognizing a definite change in the position pattern during depletion and restoration of Nissl material (Pl. 1, figs. 2, 4). A peripheral movement of the sex chromatin begins in some cells during the second day after stimulation. Progressively more cells are involved during the succeeding 4 days, the maximum migration of the sex chromatin coinciding with the maximum enlargement of the nucleolus. This is followed by a gradual reduction in the number of cells with the sex chromatin free in the nucleoplasm, with considerable variation from one animal to another, and the normal topography is usually restored by the nineteenth day. The peripheral movement of the sex chromatin falls short of the nuclear membrane. The location of the sex chromatin for normal and chromatolytic hypoglossal neurons at 3, 7 and 13 days following stimulation, and also in the unstimulated control animal, is illustrated by the histograms in Text-fig. 1.

Movement of the sex chromatin in the nucleoplasm is slow; the rate can hardly be more than a few microns daily at the most. It may be suspected that the sex chromatin moves a little more rapidly in a peripheral direction than it does when returning to the nucleolus. Beyond this supposition, we do not know how uniform the rate of movement may be or whether the movement is constant or intermittent. Neither do we know the exact proportion of cells in which movement of the sex chromatin occurs. There is a considerable number of cells on the experimental side in which the sex chromatin is at the nucleolus. In some of these cells the sex chromatin may have yet to embark on a peripheral movement or it may already have returned from such an excursion. The position of the sex chromatin in a group of control cells suggests that some movement may occur under normal conditions, involving relatively few cells at one time.

So far as we can determine, the orientation of the sex chromatin and the direction of its peripheral movement are random. The position of the sex chromatin bears no constant relationship to the polarity of the neuron, using the axon hillock as a point of reference. A particularly close analysis of the direction of movement was made in the altered cells at 1, 2 and 3 days survival time, when nuclear caps are encountered frequently (especially in the 3-day animal). The direction of peripheral movement appears again to be random, and no convincing evidence could be found of any relationship between the direction in which the sex chromatin moves and the location of nuclear caps. This suggests that the forces responsible for movement of the sex chromatin are active in all radii of the nucleus.

While the movement of the sex chromatin was being studied, we gained the impression that it enlarges slightly in chromatolytic cells. The sex chromatin was





Text-fig. 1. Histograms illustrating the position of the sex chromatin in normal (left) and chromatolytic (right) neurons of the hypoglossal nuclei at 3, 7 and 13 days following stimulation, and also in the unstimulated control animal.

measured with a filar micrometer eye-piece in 200 nuclei of control and experimental hypoglossal cell groups in the female cat of 7 days survival time. The mean diameter was  $0.8\mu$  in control cells and  $1.0\mu$  in chromatolytic cells. The difference is so slight as to be of doubtful significance taken by itself. However, since the impression of enlargement of the sex chromatin was gained from both this series and the series studied by Barr & Bertram, as well as after nerve crush and nerve section (Crouch & Barr, 1954), we felt certain that such an enlargement does occur. Crouch & Barr found that a proportion of the enlarged sex chromatin masses, following section of the hypoglossal nerve, showed some indication of a double structure, assuming the appearance of a diplococcus. This was not encountered in the present experiments.

The sex chromatin in hypoglossal neurons of male cats is so small as to be practically unrecognizable. A minute particle of chromatin, which may be the sex chromatin of the male, was seen in about 10 % of control cells in the two male cats (survival times 3 and 7 days). On the experimental side, a discrete particle of chromatin was seen free in the nucleoplasm in about 25 % of cells. This is thought to be male sex chromatin which has enlarged just enough to make identification possible, although still with some difficulty. Similar observations were made in the earlier series and following crush and section of the hypoglossal nerve. These observations have led to the view that male neurons contain sex chromatin which is so small that it lies at, or beyond, the level of resolution with standard optical equipment but which, like the female sex chromatin, may be induced to enlarge a little under experimental conditions.

The *accessory body of Cajal* was studied in protargol-stained sections from twelve animals (Table 1). In the other five cats, either insufficient material remained after the methyl green-pyronin and Feulgen preparations had been made, or difficulty was experienced in obtaining good results with the protargol method. This is a common experience when working with silver techniques.

The main characteristics of the accessory body in normal cells of the hypoglossal nucleus are as follows (Pl. 1, fig. 5). The accessory body and the sex chromatin are of approximately the same size in hypoglossal neurons, although current work shows that the accessory body is smaller than the sex chromatin in certain regions of the nervous system. Unlike the sex chromatin, there is no difference in the accessory body according to sex. The figures recorded for the single male animal in Table 1 have been confirmed in additional male cats and in other regions of the nervous system. The accessory body in both sexes and the sex chromatin in females are seen with about the same frequency in  $10\mu$  sections of normal cells which contain the nucleolus. As shown in Table 1, the accessory body is more likely to be free in the nucleoplasm than elsewhere, while the sex chromatin of the cat's hypoglossal nucleus is most often adjacent to the nucleolus. The average values for the position of the accessory body are as follows: adjacent to the nucleolus 13 %, free in the nucleoplasm 62 %, and adjacent to the nuclear membrane 1 % (total 76 %). The accessory body is spherical with a smooth contour, and no details of internal structure can be seen. It stains readily with reduced silver methods and stands out sharply against the general nuclear background in good preparations. We have seen the accessory body only in material stained by silver methods. Since it does not stain with cresyl violet, methyl green-pyronin or the

Feulgen method, it may be inferred that the accessory body lacks any appreciable amounts of nucleic acid. No other facts concerning its chemical nature or its relation to other nuclear structures appear to be known. The sex chromatin lacks well-developed argyrophilic properties. However, the sex chromatin and the accessory body of Cajal are seen in the same nucleus when a section of female tissue is stained with methyl green following preliminary staining with protargol (Pl. 1, fig. 7).

The position of the accessory body does not change, at least to any great extent, during depletion and restoration of the Nissl material under the conditions of these experiments. Although some movement of the accessory body within the nucleo-

Table 1. *Incidence and position of the accessory body of Cajal in 10 $\mu$  sections containing the nucleolus and stained with protargol*

Survival time (days)	Sex	Hypoglossal nucleus	Position of accessory body (%)			Total (%)
			Adjacent to nucleolus	Free in nucleo- plasm	Adjacent to nuclear membrane	
Control	F.	Left	9	78	1	88
		Right	12	61	2	75
1	F.	Control	10	66	1	77
		Exper.	13	57	0	70
2	F.	Control	20	60	1	81
		Exper.	22	39	0	61
4	F.	Control	22	56	2	80
		Exper.	26	41	1	68
7	F.	Control	8	48	1	57
		Exper.	16	50	4	70
7	M.	Control	26	49	0	75
		Exper.	13	42	2	57
9	F.	Control	15	62	2	79
		Exper.	8	54	2	64
11	F.	Control	9	66	0	75
		Exper.	16	39	1	56
13	F.	Control	11	74	1	86
		Exper.	13	56	1	70
15	F.	Control	7	71	0	78
		Exper.	13	65	0	78
17	F.	Control	6	62	0	68
		Exper.	4	46	2	52
18	F.	Control	14	56	0	70
		Exper.	11	56	0	67

plasm might escape notice, there is certainly not the clear peripheral movement that is shown by the sex chromatin. However, an interesting and apparently previously unobserved decrease in size of the accessory body does occur in the experimental cells (Pl. 1, fig. 6). In fact, it becomes so small in many chromatolytic cells that identification is difficult. The normal size is regained by the seventeenth or eighteenth day after stimulation.

#### DISCUSSION

On the basis of observations gathered from many sources, an explanation can be suggested for the changes in the nucleolus during depletion and restoration of the Nissl material. Much work remains to be done before the changes in the sex chromatin and the accessory body are understood.



There is much evidence in the literature to indicate that the nucleolus plays an important part in the synthesis of cytoplasmic proteins, with the ribose nucleoproteins of the cytoplasm as an intermediary (Caspersson & Schultz, 1940; Vogt & Vogt, 1947; Caspersson, 1947, 1950; Lagerstedt, 1949; Brachet, 1952). The following examples have been selected to show that this relationship between the nucleolus, cytoplasmic RNA and protein synthesis seems to be a broad biological principle.

Ludford (1922) described nucleolar extrusions into the cytoplasm during oogenesis in a mollusc and interpreted this phenomenon as being related to the synthesis of yolk. In scorpions, oocytes of some species accumulate protein-containing yolk. The nucleolus enlarges considerably during yolk formation and emits 'round basophil bodies' which appear to traverse the nuclear membrane. In other species of scorpion there is no yolk in the oocyte and little sign of nucleolar activity (Nath 1925). Gardiner (1927) described an enlargement and increased vacuolation of the nucleolus, with nucleolar emissions into the cytoplasm, during yolk formation in the oocyte of the king crab. There is a great increase in the number of nucleoli during maturation of the toad's egg, and the newly formed nucleoli make their way to the nuclear membrane (Painter & Taylor, 1942). The sequence of events suggests that the nucleoli are instrumental in the formation of cytoplasmic nucleoprotein. Callan (1952) noted a similar phenomenon in oocytes of the newt.

There are many observations which demonstrate the relationship of the nucleolus to cell growth in embryonic tissues. The studies of Hydén (1943) and LaVelle (1951) refer specifically to the neuron and indicate that the nucleolus is instrumental in the elaboration of the ribose nucleoproteins of the Nissl material. The observations of Thorell (1944) point to a similar function of the nucleolus during maturation of blood cells in the adult. In the earthworm, the nucleoli are enlarged in regions where regeneration is taking place (Sayles, 1927).

Nucleocytoplasmic relationships have been studied rather extensively in gland cells since they secrete large amounts of protein, sometimes in the form of enzymes, and since the level of activity can be altered by drugs in certain instances. Painter (1945) showed that nucleoli increase in number and size during production of royal jelly by the lateral pharyngeal gland of the worker honey-bee, and during increased activity of the salivary glands and so-called fat cells of *Drosophila*. Noback & Montagna (1947) and Yokoyama & Stowell (1951) relate the activity of the nucleolus to the production of cytoplasmic ribose nucleoproteins and enzymes in pancreatic cells, as a result of changes which occur following the injection of pilocarpine. The latter authors, in particular, noted the enlargement and vacuolation of the nucleoli which takes place during the formation of cytoplasmic nucleoprotein and the synthesis of enzymic proteins. Payne (1942) has described a considerable enlargement of nucleoli in the basophiles of the fowl's pituitary as these cells increase in number and size after castration.

The neuron has been a favourite cell for the study of nucleocytoplasmic relationships because of the large size of many neurons, the large nucleolus and the abundant ribose nucleoprotein of the Nissl material. Support for the conception that the nucleolus participates in the synthesis of Nissl material has been recorded by Einarson (1933), Hydén (1943, 1947), Hamburger & Hydén (1945, 1949*a*, *b*), and a large number of other authors over a period of several decades. A few have been

reluctant to accept such an interpretation (Andrew & Andrew, 1942; Gersh & Bodian, 1943; Bodian, 1947).

Evidence for increased activity on the part of the nucleolus has also been claimed for the abnormal growth of cancer cells (MacCarty, 1936; Caspersson & Santesson, 1942).

In the light of the preceding reports, and many others which have not been mentioned, we have arrived at the following interpretation of the observations here recorded. Depletion of the Nissl material initiates a series of events which seems designed to restore the amount of Nissl material which is normal for the cell. Although one could point to exceptions, in general the changes in the cytoplasm suggest that the Nissl material is restored from the nucleus toward the periphery. The nuclear caps, which have an intimate relationship with the nuclear membrane, can hardly be other than newly formed Nissl material. In later stages of recovery, the perinuclear zone contains basophilic material in relatively large amounts. The nucleus is thus implicated in the synthesis of Nissl material, although this by no means excludes the coincident cytoplasmic reactions which undoubtedly occur and are directed toward the same end.

An interval of a day or a little more elapses between the beginning of depletion of the Nissl material and swelling and vacuolation of the nucleolus. The greatest changes in the nucleolus coincide with the period of restoration of the Nissl material. It is inferred from these relationships that depletion of the Nissl material serves as a stimulus to the nucleolus, causing an increased activity on the part of this organelle directed toward restoration of the Nissl material. The swelling and vacuolation of the nucleolus are regarded as a morphological expression of increased activity. The accelerated mechanisms for the production of Nissl material gather momentum, once having been set in motion, and cause a transient hyperchromasia of the cytoplasm. The increased demands on the nucleolus subside as the normal complement of Nissl material is attained or exceeded, and its normal morphology is gradually restored. The behaviour of the nucleolus of a chromatolytic neuron is relatively unspectacular, but none the less significant, compared with the multiplication of nucleoli, their swelling and vacuolation and their peripheral migration and even extrusion into the cytoplasm which occurs during the growth period of oocytes and secretory phases of certain gland cells in insects. We feel, therefore, that there is a close interrelationship between the nucleolus and the ribose nucleoproteins of the Nissl material, the mechanisms of nucleoprotein synthesis being in a relatively steady state under normal conditions.

Caspersson and his collaborators describe a diffuse 'chromocentre' located near the nucleolus and visible in ultra-violet photomicrographs and after staining with acid dyes. The chromocentre contains proteins of a special type, DNA, and RNA. The chromocentre and the nucleolus are considered to be closely related functionally and are together called the nucleolar apparatus. The stains which we have used are apparently not competent to show such a chromocentre, if it is present in the cells we have studied. The chemical reactions involved in the nucleolar-cytoplasmic relationship are largely unknown. Caspersson and his collaborators have demonstrated that proteins containing large amounts of hexone bases and diamino acids flow from the nucleolar apparatus to the nuclear membrane, and suggest that they participate in

some way in the elaboration of RNA and cytoplasmic proteins. The nucleolus may be involved in the synthesis of cytoplasmic materials other than nucleoproteins, although such possible aspects of its function have received little attention. So far as the function of the Nissl material is concerned, the view of Hydén is the most plausible at the present time; namely, that large amounts of ribose nucleoprotein are required to maintain the cytoplasm of the neuron, certain types of neuron being the largest uninuclear cells in the body when the volume of the processes is included.

The movement of the sex chromatin during chromatolysis is a fascinating phenomenon which we are at a loss to explain in view of the paucity of information on the forces acting on nuclear structures. A discussion of this movement is unquestionably premature and frankly speculative, yet it may serve a useful purpose in relation to future work. The following possibilities suggest themselves for consideration.

The sex chromatin could be carried along in the flow of materials from the nucleolus toward the nuclear membrane. However, there must be a comparable flow of materials in the reverse direction; in fact, experiments of this nature emphasize the magnitude of the to-and-fro traffic which must occur across the nuclear membrane, even allowing for its active participation in the metabolism of the cell. For the movement of the sex chromatin to be produced in this way, it is necessary to postulate a predominant flow from nucleolus to nuclear membrane in a certain sector of the nucleus, with the flow in the reverse direction predominating elsewhere. Such an arrangement seems to exist in spinal ganglion cells of the fish (Holmgren, 1899; Hydén, 1943), but there is no good evidence for this in the cells under discussion. The return of the sex chromatin to the nucleolus is not accounted for in this way. Further, there are reasons for thinking that forces originating at the periphery of the nucleus may be more influential than forces emanating from the nucleolus. The peripheral movement of the sex chromatin precedes slightly the changes on the nucleolus, although the interval may not be large enough to be significant. Further, Crouch & Barr (1954) found that the sex chromatin remains free of the nucleolus, following nerve section, long after the normal size and appearance of the nucleolus have been restored. Although the centrifugal flow of materials of nucleolar origin may be a factor in the outward movement of the sex chromatin, it appears unlikely that this mechanism is alone responsible.

Viscosity changes in the nucleolus may be implicated. The viscosity of the nucleoplasm is probably not very high in these large vesicular nuclei. It is conceivable that the sex chromatin might be carried along at the advancing edge of a wave of gelation proceeding outwards from the nucleolus. It is more difficult to visualize a similar process occurring in the reverse direction, to carry the sex chromatin to its usual position against the nucleolus. Further, the rate of movement seems much too slow to be explicable on the basis of a sol-gel transformation.

The possibility that the movement of the sex chromatin is caused by altered electrostatic forces remains to be considered. Judging from its behaviour in an electrical field, the nucleus as a whole is said to have a positive charge, and the chromosomes a negative charge (Hardy, 1913; Churney & Klein, 1937). De Robertis, Nowinski & Saez (1949) state that the nuclear membrane has a positive charge. They point out that chromatin is an ampholyte, with both positive and negative



charges, and that its behaviour with respect to an external charge depends on the pH of the medium. There may be a change in the pH of the nucleoplasm during altered metabolic states of the cell, in addition to whatever changes may occur in the potential on the nuclear membrane, nucleolus and sex chromatin. A body within a sphere is subjected to equal electrostatic forces in all directions, regardless of its position, if the wall of the sphere has a uniform potential throughout. In order to consider altered electrical forces acting from the nuclear membrane as important in causing movement of the sex chromatin, it is necessary to postulate a non-uniform potential on the nuclear membrane, although such a requirement is subject to modification in view of the presence of a large nucleolus which probably has an electrical charge. This and other difficulties arise in considering electrostatic forces, yet we feel that such a hypothesis is worthy of consideration. The foregoing speculative discussion does little more than lay bare the need for information concerning the physical forces which operate within the nucleus.

In addition to remaining Feulgen-positive, the sex chromatin has the same affinity for methyl green in chromatolytic and normal neurons, so far as this can be judged without the use of quantitative methods. According to the work of Kurnick (1952*b* and earlier), this indicates that there is no extensive depolymerization of the DNA of the sex chromatin in chromatolytic cells.

The sex chromatin, as mentioned earlier, is thought to represent heterochromatic regions of the sex chromosomes, which probably contain large numbers of genes of the same type and therefore exhibit a uniform behaviour with respect to nucleic acid metabolism (Pontecorvo, 1944). Heterochromatic regions tend to remain compact in the resting nucleus and retain a high concentration of DNA. The sex chromosomes exhibit this behaviour to a special degree. There is considerable evidence, arising from observations on the most varied cell types, that heterochromatic regions of chromosomes share with the nucleolus a controlling influence over the synthesis of cytoplasmic RNA and protein (Schultz & Caspersson, 1939; Painter & Taylor, 1942; White, 1943; Painter, 1945; Koller, 1947; Hydén, 1947; Caspersson, 1947, 1950). This may be the explanation for such enlargement of the sex chromatin as occurs during the accelerated synthesis of Nissl material. If techniques of sufficient accuracy were available, it is conceivable that a sex difference in the rate of nucleoprotein synthesis, in favour of the female, might be detected.

The observations on the accessory body of Cajal are of a preliminary nature, and further studies of this interesting structure are in progress. A more precise analysis of its behaviour during chromatolysis may help to clarify the nature of the forces which are responsible for movement of the sex chromatin. If it is established definitely that the accessory body remains quite stationary while the sex chromatin moves, this would be a point in favour of the hypothesis of electrostatic forces, in view of the difference in the chemical composition and, in all probability, the electrical charge on the two structures. Such evidence would carry more weight were the accessory body, like the sex chromatin, adjacent to the nucleolus in the majority of normal cells. The decrease in size of the accessory body, assuming that the reduced silver method gives a faithful representation of this, is inexplicable since virtually nothing is known of its composition or role in cell metabolism.

Brusa (1952) feels that the sex chromatin should be considered as a basophile clot

of Levi when it is adjacent to the nucleolus, and as an accessory body of Cajal when it is free of the nucleolus. We believe this to be unwarranted for the following reasons. The basophile clots of Levi are always at the rim of the nucleolus and are found most typically in nerve cells of rodents. Unlike the sex chromatin, a relationship between the size of the basophile clots and the sex of the animal has not been demonstrated. In nerve cells of certain other mammals, such as the monkey, there are perinucleolar masses of chromatin which probably qualify for inclusion under the term basophile clots of Levi. However, there is little difficulty in distinguishing the sex chromatin from these perinucleolar masses in the monkey, since the sex chromatin is well developed in females only, where it is most likely to be found against the nuclear membrane (Prince, 1952). It is important, therefore, to distinguish the sex chromatin from perinucleolar masses of chromatin such as the basophile clots of Levi, which are not known to have a relationship to sex.

During the initial phase of our study of the sex chromatin, it was thought that the sex chromatin and the accessory body of Cajal were the same structure (Barr, Bertram & Lindsay, 1950). A more searching analysis has shown this supposition to be incorrect for the following reasons. The accessory body has the same size in both sexes and its topography differs from that of the sex chromatin. The accessory body is strongly argyrophilic and refractory to basic dyes; the reverse is true for the sex chromatin. Finally, it is not difficult to see the accessory body and the sex chromatin in the same nucleus if the section is stained with protargol, followed by a basic dye such as methyl green. Although the sex chromatin was undoubtedly seen by most neurocytologists and thought to correspond with other nuclear structures, it is now important that the sex chromatin be recognized as a unique component of the nucleus and the one which is most intimately related to the sex chromosomes.

#### SUMMARY

1. The behaviour of the nucleolus, sex chromatin and accessory body of Cajal was studied during depletion and restoration of the Nissl material in neurons of the cat's hypoglossal nucleus. Changes in the nucleolus were followed in preparations stained with methyl green-pyronin, since the nucleolus stains with pyronin because of its content of RNA. The sex chromatin contains DNA and is stained with methyl green and by the Feulgen method. Reduced silver methods are required to demonstrate the argyrophilic accessory body. This is an extension of earlier work reported in this *Journal* by Barr & Bertram (1951), who studied similar preparations after staining with cresyl violet.

2. Swelling and vacuolation of the nucleolus occur during the period of most active restoration of the Nissl material. These changes are regarded as a morphological indication of increased activity on the part of the nucleolus. It is concluded that the nucleolus participates in the synthesis of the ribose nucleoproteins of the Nissl material.

3. The sex chromatin of many neurons moves from the nucleolus toward the nuclear membrane during depletion and restoration of the Nissl material. There is a slight increase in the size of the sex chromatin during the same period. These observations are of interest since the sex chromatin appears to represent hetero-

chromatic regions of the sex chromosomes, and since there is evidence from other sources that heterochromatic chromosome regions participate in nucleocytoplasmic interactions of a synthetic nature.

4. The accessory body of Cajal, as seen in protargol preparations, decreases in size in chromatolytic neurons, but there is no obvious change in its position in the nucleus. So far as we are aware, the nature of the accessory body and whatever function it may have in the physiology of the cell are unknown.

5. It is important to distinguish clearly between the sex chromatin and other components of the nucleus, such as the basophile clots of Levi and the accessory body of Cajal.

This work was made possible by grants-in-aid to one of the authors (M. L. B.) from the National Cancer Institute and National Health Grants (Mental Health Division) of Canada. Mr J. E. Walker and Mr C. E. Jarvis gave valuable technical assistance.

#### REFERENCES

- ANDREW, W. & ANDREW, N. L. (1942). The chromatin content of nerve cells in man and in the mouse with special regard to the role of the nucleolus; observations in normal and malnourished specimens. *J. comp. Neurol.* **76**, 423-433.
- BARR, M. L. (1954). An interim note on the application of the skin biopsy test of chromosomal sex to hermaphrodites. *Surg. Gynec. Obstet.* **99**, 184-186.
- BARR, M. L. & BERTRAM, E. G. (1951). The behaviour of nuclear structures during depletion and restoration of Nissl material in motor neurons. *J. Anat., Lond.*, **85**, 171-181.
- BARR, M. L., BERTRAM, L. F. & LINDSAY, H. A. (1950). The morphology of the nerve cell nucleus, according to sex. *Anat. Rec.* **107**, 283-298.
- BODIAN, D. (1947). Nucleic acid in nerve cell regeneration. *Symp. Soc. exp. Biol.* **1**, 163-178.
- BRACHET, J. (1952). The role of the nucleus and the cytoplasm in synthesis and morphogenesis. *Symp. Soc. exp. Biol.* **6**, 173-200.
- BRUSA, A. (1952). A propos de la structure du noyau de la cellule nerveuse. *Anat. Anz.* **98**, 343-352.
- CALLAN, H. G. (1952). A general account of experimental work on amphibian oocyte nuclei. *Symp. Soc. exp. Biol.* **6**, 243-255.
- CAMPBELL, B. & NOVICK, R. (1946). A quantitative method for the study of chromatolysis. *Proc. Soc. exp. Biol., N.Y.*, **61**, 425-427.
- CASPERSSON, T. (1947). The relations between nucleic acid and protein synthesis. *Symp. Soc. exp. Biol.* **1**, 127-151.
- CASPERSSON, T. (1950). *Cell Growth and Cell Function*. New York: W. W. Norton and Co. Inc.
- CASPERSSON, T. & SANTESSON, L. (1942). Studies on protein metabolism in the cells of epithelial tumours. *Acta radiol., Stockh.*, (Suppl.), **46**, 1-105.
- CASPERSSON, T. & SCHULTZ, J. (1940). Ribonucleic acids in both nucleus and cytoplasm, and the function of the nucleolus. *Proc. nat. Acad. Sci., Wash.*, **26**, 507-515.
- CHURNEY, L. & KLEIN, H. M. (1937). The electrical charge on nuclear constituents (salivary gland cells of *Sciara coprophilia*). *Biol. Bull., Woods Hole*, **72**, 384-388.
- CONN, H. J. & DARROW, M. A. (1943). *Staining Procedures*. Geneva, N.Y.: Biotech Publications.
- CROUCH, Y. F. & BARR, M. L. (1954). The behaviour of the sex chromatin during axon reaction. *J. Neuropath.* **13**, 353-358.
- DE ROBERTIS, E. D. P., NOWINSKI, W. W. & SAEZ, F. A. (1949). *General Cytology*. Philadelphia: W. B. Saunders Co.
- EINARSON, L. (1933). Notes on the morphology of the chromophil material of nerve cells and its relation to nuclear substances. *Amer. J. Anat.* **53**, 141-175.
- GARDINER, M. S. (1927). Oogenesis in *Limulus polyphemus*, with especial reference to the behaviour of the nucleolus. *J. Morph.* **44**, 217-264.
- GERSH, I. & BODIAN, D. (1943). Some chemical mechanisms in chromatolysis. *J. cell. comp. Physiol.* **21**, 253-279.



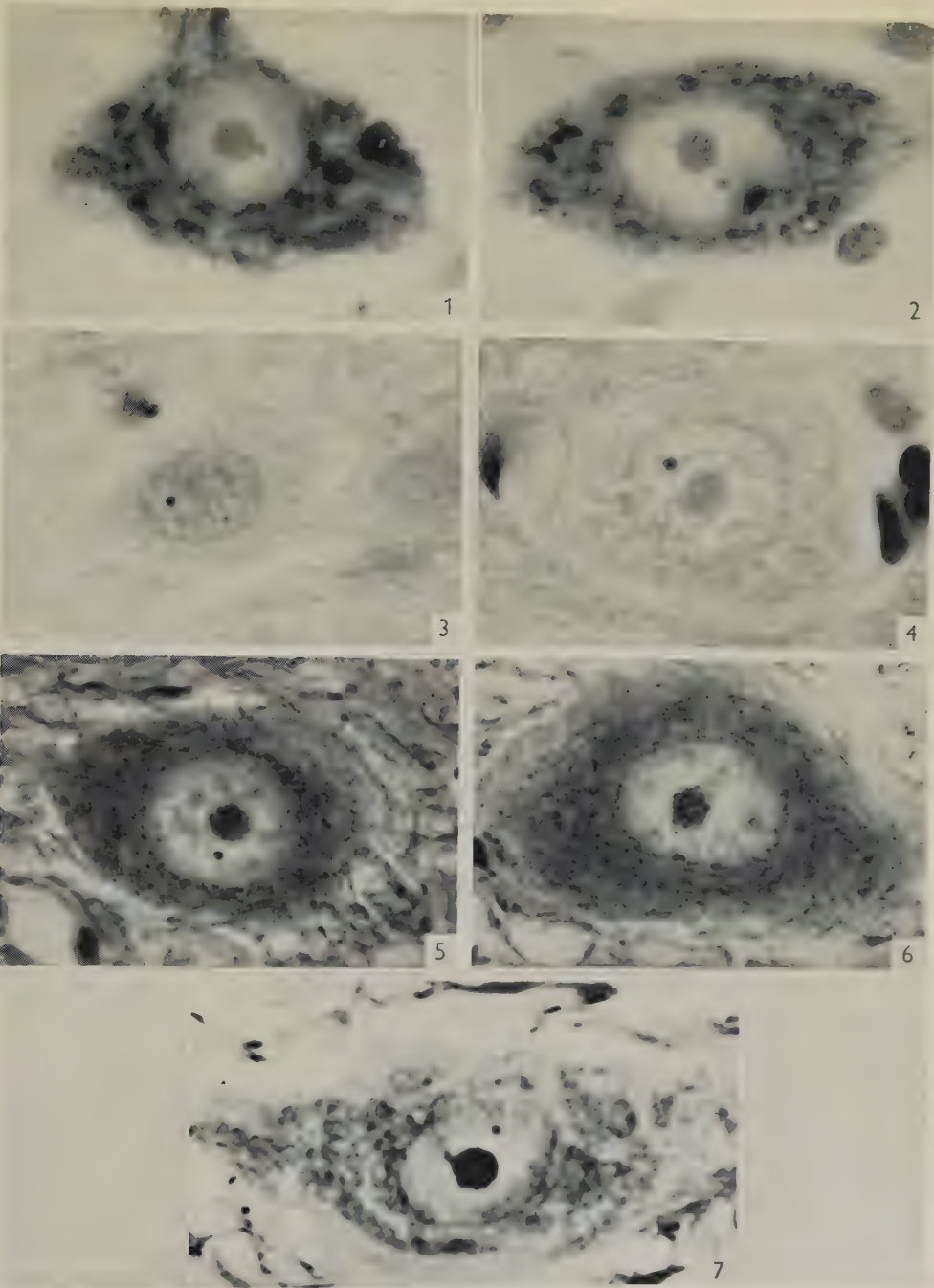
- GRAHAM, M. A. (1954a). Detection of the sex of cat embryos from nuclear morphology in the embryonic membrane. *Nature, Lond.*, **173**, 310-311.
- GRAHAM, M. A. (1954b). Sex chromatin in cell nuclei of the cat from the early embryo to maturity. *Anat. Rec.* (in the Press).
- GRAHAM, M. A. & BARR, M. L. (1952). A sex difference in the morphology of metabolic nuclei in somatic cells of the cat. *Anat. Rec.* **112**, 709-723.
- HAMBURGER, C. A. & HYDÉN, H. (1945). Cytochemical changes in the cochlear ganglion caused by acoustic stimulation and trauma. *Acta oto-laryng., Stockh.*, (Suppl.), **61**, 1-89.
- HAMBURGER, C. A. & HYDÉN, H. (1949a). Production of nucleoproteins in the vestibular ganglion. *Acta oto-laryng., Stockh.*, (Suppl.), **75**, 53-81.
- HAMBURGER, C. A. & HYDÉN, H. (1949b). Transneuronal changes in Deiter's nucleus. *Acta oto-laryng., Stockh.*, (Suppl.), **75**, 82-113.
- HARDY, W. B. (1913). Notes on differences in electrical potential within the living cell. *J. Physiol.* **47**, 108-111.
- HOLMGREN, E. (1899). Zur Kenntnis der Spinalganglienzellen von *Lophius piscatorius* Lin. *Anat. Hefte*, **38**, 71-154.
- HYDÉN, H. (1943). Protein metabolism in the nerve cell during growth and function. *Acta physiol. scand.* (Suppl.), **17**, 1-136.
- HYDÉN, H. (1947). Protein and nucleotide metabolism in the nerve cell under different functional conditions. *Symp. Soc. exp. Biol.* **1**, 152-162.
- KOLLER, P. C. (1947). The experimental modification of nucleic acid systems in the cell. *Symp. Soc. exp. Biol.* **1**, 270-290.
- KURNICK, N. B. (1950). Methyl green-pyronin. 1. Basis of selective staining of nucleic acids. *J. gen. Physiol.* **33**, 243-264.
- KURNICK, N. B. (1952a). Histological staining with methyl green-pyronin. *Stain Tech.* **27**, 233-242.
- KURNICK, N. B. (1952b). The basis of specificity of methyl green staining. *Exp. Cell. Res.* **3**, 649-651.
- LAGERSTEDT, S. (1949). Cytological studies on the protein metabolism of the liver in rat. *Acta anat.* (Suppl.), **9**, 1-116.
- LAVELLE, A. (1951). Nucleolar changes and development of Nissl substance in the cerebral cortex of fetal guinea pigs. *J. comp. Neurol.* **94**, 453-473.
- LUDFORD, R. J. (1922). The morphology and physiology of the nucleolus. *J. R. micr. Soc.* pp. 113-150.
- MACCARTY, W. C. (1936). The value of the macronucleus in the cancer problem. *Amer. J. Cancer*, **26**, 529-532.
- MOORE, K. L. & BARR, M. L. (1953). The morphology of the nerve cell nucleus in mammals, with special reference to the sex chromatin. *J. comp. Neurol.* **98**, 213-231.
- MOORE, K. L. & BARR, M. L. (1954). Nuclear morphology, according to sex, in human tissues. *Acta anat.* **21**, 197-208.
- MOORE, K. L., GRAHAM, M. A. & BARR, M. L. (1953). The detection of chromosomal sex in hermaphrodites from a skin biopsy. *Surg. Gynec. Obstet.* **96**, 641-648.
- NATH, V. (1925). Cell inclusions in the oogenesis of scorpions. *Proc. Roy. Soc. B*, **98**, 44-58.
- NOBACK, C. R. & MONTAGNA, W. (1947). Histochemical studies of the basophilia, lipase and phosphatases in the mammalian pancreas and salivary glands. *Amer. J. Anat.* **81**, 343-367.
- PAINTER, T. S. (1945). Nuclear phenomena associated with secretion in certain gland cells with especial reference to the origin of cytoplasmic nucleic acid. *J. exp. Zool.* **100**, 523-547.
- PAINTER, T. S. & TAYLOR, A. N. (1942). Nucleic acid storage in the toad's egg. *Proc. nat. Acad. Sci., Wash.*, **28**, 311-317.
- PAYNE, F. (1942). The cytology of the anterior pituitary of the fowl. *Biol. Bull., Woods Hole*, **82**, 79-111.
- PONTECORVO, G. (1944). Structure of heterochromatin. *Nature, Lond.*, **153**, 365-367.
- PRINCE, R. H. (1952). Sex and the Cell Nucleus. M.Sc. Thesis, University of Western Ontario.
- RAMÓN Y CAJAL, S. (1910). El núcleo de las células piramidales (del cerebro humano y de algunos mamíferos). *Trab. Lab. Invest. biol. Univ. Madr.* **8**, 27-62.
- SAYLES, L. P. (1927). Origin of the mesoderm and behaviour of the nucleolus in regeneration in *Lumbriculus*. *Biol. Bull., Woods Hole*, **52**, 278-311.

- SCHULTZ, J. & CASPERSSON, T. (1939). Heterochromatic regions and the nucleic acid metabolism of the chromosomes. *Arch. exp. Zellforsch.* **22**, 650-654.
- STOWELL, R. E. (1945). Feulgen reaction for thymonucleic acid. *Stain Tech.* **20**, 45-58.
- TAFT, E. B. (1951). The specificity of the methyl green-pyronin stain for nucleic acids. *Exp. Cell Res.* **2**, 312-326.
- THORELL, B. (1944). Behaviour of the nucleolar apparatus during growth and differentiation of the normal blood cells in the adult stage. *Acta med. scand.* **117**, 334-375.
- VOGT, C. & VOGT, O. (1947). Lebensgeschichte, Funktion und Tätigkeitsregulierung des Nucleolus. *Theoret. Med.* **1**, 43-50.
- WHITE, M. J. D. (1943). Amount of heterochromatin as a specific character. *Nature, Lond.*, **152**, 536-537.
- YOKOYAMA, H. D. & STOWELL, R. E. (1951). Nucleolar volume changes in the mouse pancreas after repeated pilocarpine injections. *J. nat. Cancer Inst.* **11**, 939-945.

#### EXPLANATION OF PLATE

All photomicrographs show nerve cells of *female* cats. Magnification  $\times 1200$ .

- Fig. 1. Normal neuron in the left hypoglossal nucleus, methyl green-pyronin stain. The sex chromatin is adjacent to the nucleolus. This is its most common position in normal neurons of the cat's hypoglossal nucleus. The sex chromatin is green in the actual preparation, while the nucleolus and Nissl material are red.
- Fig. 2. A nerve cell of the right hypoglossal nucleus in a state of moderately severe chromatolysis, stained with methyl green-pyronin. The slightly enlarged sex chromatin is free in the nucleoplasm. Its affinity for methyl green is unimpaired. The dense area at the nuclear membrane is a nuclear cap, which is thought to represent newly synthesized Nissl material.
- Fig. 3. Normal neuron in the left hypoglossal nucleus stained by the Feulgen method and an Orange-G counterstain. The Feulgen-positive sex chromatin is adjacent to the nucleolus.
- Fig. 4. An experimentally altered neuron of the right hypoglossal nucleus, stained by Feulgen and Orange-G. The slightly enlarged sex chromatin in the nucleoplasm retains its Feulgen-positive staining property.
- Fig. 5. Normal nerve cell of the left hypoglossal nucleus stained with protargol. The accessory body of Cajal is shown in its usual location, i.e. free in the nucleoplasm. Unlike figs. 1-4, this illustration would serve equally well for a male neuron.
- Fig. 6. Experimentally altered neuron of the right hypoglossal nucleus, stained with protargol. The accessory body, which is much smaller than normal, lies a short distance from the nucleolus, at about 7 o'clock.
- Fig. 7. A normal nerve cell in the hypoglossal nucleus stained first with protargol and then with methyl green. In the actual preparation the accessory body, which is free in the nucleoplasm, and the nucleolus are brown (protargol), while the sex chromatin next to the nucleolus is green (methyl green).



LINDSAY AND BARR—RELATION OF NUCLEUS TO NISSL MATERIAL





# ENUMERATION OF GLOMERULI IN THE KIDNEY OF THE DOG

BY R. V. SELLWOOD\* AND E. B. VERNEY

*University Department of Pharmacology, Cambridge*

In the literature there is discrepancy in the figures for the number of glomeruli in the kidney of the dog as estimated by different methods. Brodie & Thackrah (1914) found 125,000 and 142,000 glomeruli per kidney by a histological computation, while other observers using various maceration techniques have arrived at figures around 500,000 per kidney for a dog of average size (Vimtrup, 1928; Kunkel, 1930). Even when the same technique is used by a single observer there is a wide range of variation in the number of glomeruli found in the kidneys of different dogs. In Kunkel's (1930) series this range was from 160,000 to 726,000 per kidney.

Provided that a histological glomerular count is made on a proportion of material that is sufficiently large to ensure its being representative of the whole kidney, it theoretically gives the most reliable means of determining the total number of glomeruli present. The time involved in such a determination is, however, so very much longer than that occupied by a maceration technique that it becomes important to know whether the two procedures applied to the kidneys of the larger mammals yield answers which are in sufficiently close agreement to warrant confidence in the maceration method. We have, therefore, compared the results of these two methods when applied to specimens of the same renal tissue suitably fixed for histological investigation. It was felt that a more reliable estimate of the average number of glomeruli per gram of cortex as determined by serial sectioning of tissue samples would be obtained if the kidney were immediately fixed throughout by perfusion *in situ* than if it were excised and placed in the fixative solution, and that maceration, too, would proceed more uniformly under these circumstances.

## METHODS

### *Fixation of the kidneys*

These were fixed *in situ* in the following way. A dog (wt. 19.8 kg.) was anaesthetized by an intravenous injection of chloralose (0.1 g./kg. body wt.), and then eviscerated by successive division between ligatures of the coeliac axis, superior and inferior mesenteric arteries, rectum, cardiac end of the stomach and the structures entering the portal fissure. The kidneys and the renal segments of the aorta and inferior vena cava were then freed by dividing between ligatures the peritoneal attachments of the kidneys, and the lumbar vessels. Loose ligatures were placed around the freed segments of the aorta and vena cava well anterior and well posterior to the renal vessels, and the aorta and vena cava were tied posterior to the lower ligatures. Bull-dog clips were then applied to the aorta and vena cava immediately posterior to the renal vessels, and cannulae were inserted below the clips. The aortic cannula,

\* Scholar of the Australian National University, Canberra.

previously filled with 0.9 % NaCl, was connected by T-piece and rubber tubing (each tube being provided with a screw-clamp) with two Mariotte bottles placed at a height of 170 cm., the one containing 0.9 % NaCl, the other 'Susa' fluid. The caval cannula was connected with a length of wide rubber tubing that drained into an open vessel. With the aortic cannula in communication with the saline reservoir, the ligatures at the anterior ends of the renal segments of the aorta and vena cava were quickly tied and the bull-dog clips immediately removed. The flow of saline was rapid, and when the effluent was fairly free from blood the aortic cannula was switched to the 'Susa' reservoir. This fluid flowed rapidly through the kidneys and they became immediately and uniformly hardened. When about 250 ml. had passed through, the kidneys were removed and, after all adherent tissue and the hilum structures had been dissected away, each kidney was weighed and preserved in 'Susa' fluid.

*Enumeration of glomeruli by a histological technique*

With the right kidney the following procedure was adopted. A numbered series of covered Petri dishes was taken, each containing several sheets of filter paper soaked in 'Susa' fluid, and on the paper was placed a glass slide. The kidney was bisected transversely, and one 'half' was then split longitudinally, i.e. by an incision passing around the longer circumference, so that the kidney was divided as symmetrically as possible into one 'half' and two 'quarters'. These three pieces were weighed separately, and the 'half' and one 'quarter' were replaced in 'Susa' fluid. The remaining 'quarter' was thereupon cut radially into fourteen pieces: each slice thus tapered on the one hand towards its hilum end and on the other towards a point which was originally at the centre of the lateral border of the kidney. As each slice was cut it was rapidly transferred to the glass slide in one of the Petri dishes and the lid replaced. The numbering of the slices was in ascending order towards the polar region of the kidney. Later the major portion of the medulla of each slice was separated carefully from the cortex so that only a small amount of medulla remained attached, and all glomeruli were included in the cortex portion. The safety of this macroscopic separation was tested in three instances by making serial sections of weighed pieces of separated medulla and finding that they contained no glomeruli. From the centre of each slice of cortex a small sample was cut involving its whole depth, transferred rapidly to a tared weighing bottle, weighed, and blocked in paraffin. These blocks were labelled serially C 1, C 2, etc. In addition, from near each end of each of two of the slices (corresponding with the lateral aspect of the kidney and the hilum end of the slice) a sample was taken and treated in the same way: these were labelled L 1, L 2 and H 1, H 2. Any procedure that involved exposing the tissue to air before weighing, was performed as rapidly as possible. From each of the original slices a piece of cortex similar to C 1, C 2, etc., was taken, and used to determine, first, the change in weight that occurred when these pieces of tissue were kept in an atmosphere with the vapour pressure of 'Susa' fluid and under conditions identical with those imposed on the original slices, and secondly, the rate of weight loss when the pieces were exposed to air at room temperature and humidity. The remaining pieces of cortex and medulla were collected into two tared weighing bottles and weighed, so that the total weight loss in obtaining the weighed pieces for section became known.



Serial sections at  $15\mu$  were made from all the embedded pieces of tissue, the plane of section being radial so that each section involved the whole thickness of the cortex and included any attached medullary tissue. The sections were stained with haematoxylin and eosin. In all they numbered about 3000, and represented about 6.0 % of the cortex of the 'quarter' kidney.

Every third section (1, 4, 7, etc.) was then projected on to paper of uniform quality and thickness. The outline of the tissue and of each glomerulus was carefully traced, and the limit of the true cortex, i.e. the glomerulus-bearing area, was marked off from the medulla remnant. When all sections had been traced in this way, the drawings were superimposed in order over an illuminated ground-glass screen, and the glomeruli traced and numbered from one drawing to the next. The glomeruli rarely exceed  $200\mu$  in diameter, so if a glomerulus appeared to continue through six or more drawings the appearance was taken to represent two glomeruli superimposed: whenever doubt arose the intervening sections were examined. The total number of glomeruli in each block was thus found.

The drawings were cut along the tissue outlines and along the lines separating true cortex from medulla remnant. The relative weights of true cortex and medulla in each block of tissue were then calculated by weighing the portions of the drawings in each class, and the mean proportion was used for calculating the weight of true cortex in the remainder of the sliced tissue which had not been embedded. Thus the average number of glomeruli per gram of true cortex, the weight of true cortex in the whole kidney, and so the total number of glomeruli became known.

With the left kidney a somewhat similar procedure was adopted and carried through completely by another observer. In this instance, however, the slicing of the 'quarter' kidney was in a transverse direction in the sense that the thick ends of the slices were at the longitudinal circumference, and each slice tapered uniformly towards the hilum. The pieces from about the middle of the slices were labelled M 1, M 2, etc., and from the ends of two of the slices were taken two further samples. Those from the thick end were labelled B 1 and B 2, those from the thin end H 1 and H 2. The observations on this kidney were made before those on the right kidney, and at that time the stringency of the precautions needed to prevent loss of weight was not recognized. During the macroscopic sectioning and after the small pieces for paraffin embedding had been removed to tared weighing bottles, the slices were not, as was the case with the right kidney, preserved in an atmosphere with the vapour pressure of 'Susa' fluid. The needed correction for evaporative loss of weight is therefore likely to be greater than that determined for the left kidney, and the glomerular count correspondingly higher. With the left kidney no figure for this correction was obtained, and the one found for the right kidney has been applied.

#### *Enumeration of glomeruli by a maceration technique*

Kunkel's (1930) method was modified for the fixed tissue as follows. The 'quarter' kidney remaining from the histological investigation was cut into small pieces 1–2 mm. thick and placed in hydrochloric acid (sp.gr. 1.18) 50 % (v/v). The pieces were left in the acid for 1 month and then transferred to distilled water and left for 3 days. The tissue was then pulped and pipetted into a 2 l. volumetric flask. Small fragments of pelvis and kidney capsule were removed from the suspension, care

being taken that no parenchyma adhered to the pieces removed. The macerate was made up to volume with distilled water, and after it had been thoroughly mixed the glomeruli in 2 ml. portions of suspension were counted under the low power of the microscope. The counting slide was made from a piece of Perspex  $7.5 \times 7.5$  cm. and about 2 mm. thick. It was ruled vertically with parallel lines at a spacing of about three-quarters of the diameter of the low-power field. In the other direction the ruling consisted of two lines only that divided the vertical ruling into three roughly equal lengths of 2.5 cm. This slide was placed on the mechanical stage of the microscope with the closely ruled lines passing in a plane vertical to the eye of the observer, and the 2 ml. portion of suspension was pipetted on to the portion of the slide that was within the two transverse rulings. Because of the non-wetting surface, the fluid did not spread unduly, and the glomeruli and fragments of tubule soon settled so that the slide could be moved without their being displaced. The ruling allowed an accurate count of all the glomeruli in each 2 ml. sample of suspension. For the most part the glomeruli were seen with intact capsules and were well preserved. In some instances there was fragmentation of the tufts, and here a glomerulus was counted if half or more of the tuft could be seen. Altogether counts were made on 30 ml. of the suspension. The number of glomeruli in the piece of macerated kidney was thus computed and, as its weight and that of the whole kidney were known, an estimate was obtained of the number of glomeruli in that kidney.

## RESULTS

### *Histological method: right kidney*

There was a loss of weight of 2.5 % during macroscopic sectioning of the 'quarter' kidney so that the estimate must be too large by approximately this amount. The weight of the small 'control' pieces of kidney left in an atmosphere with the vapour pressure of 'Susa' fluid had not changed after a period of 12 hr. When, however, the same pieces were exposed to air and handling the loss after 200 min. was 36.8 % of the initial weight. The weight loss was linear with time. The results of the glomerular count are given in Table 1. The pieces are numbered in ascending order towards the pole of the kidney. As already explained, pieces L 1 and L 2 are from areas nearer the lateral border of the kidney, and pieces H 1 and H 2 from areas nearer the hilum than are the other pieces (C 1, C 2, etc.), these last being from positions midway between the lateral and hilum ends of the slices. The average number of glomeruli in each gram of true cortex was 18,309. The weight of the fixed kidney was 55.53 g., and the total weight of true cortex was estimated as 36.58 g. The total number of glomeruli was therefore 669,743 which on correction for the 2.5 % evaporation loss becomes 653,000.

### *Histological method: left kidney*

Here the same histological procedure had been adopted as with the right kidney but, as already explained, no stringent precautions against loss in weight during macroscopic sectioning were taken, and the same correction for this loss was applied as with the right kidney. The results are given in Table 1. As was expected, the figure for the total number of glomeruli (676,600) is greater than that found with the right kidney (653,000); and the latter figure is evidently the more reliable.

Table 1. Enumeration of glomeruli in the two kidneys of a dog

Right kidney: wt. after fixation *in situ* 55.43 g. Left kidney: wt. after fixation *in situ* 51.30 g.

Histological method			Histological method		
Piece	No. of glomeruli counted	No. of glomeruli/g. true cortex	Piece	No. of glomeruli counted	No. of glomeruli/g. true cortex
C 1	418	21,080	M 1	225	21,090
C 2	487	22,350	M 2	300	20,136
C 3	493	22,803	M 3	483	22,320
C 4	894	19,359	M 4	346	22,040
C 5	837	19,613	M 5	539	19,832
C 6	739	19,510	M 6	389	21,299
C 7	401	19,316	M 7	404	21,605
C 8	529	17,914	M 8	440	21,570
C 9	400	17,645	M 9	419	20,150
C 10	612	18,171	M 10	432	23,135
C 11	325	15,940	M 11	391	23,596
C 12	439	15,120	M 12	487	23,497
C 13	328	13,042	M 13	474	24,569
C 14	559	16,655	M 14	708	24,030
L 1	677	19,324	B 1	1,037	17,830
L 2	254	18,955	B 2	844	17,143
H 1	637	16,090	H 1	—	—
H 2	311	16,181	H 2	353	26,643
Wt. of true cortex in the sections		0.5098 g.			0.3944 g.
No. of glomeruli counted		9,340			8,321
Average number of glomeruli/g. true cortex		18,309			21,100
Ditto, corrected for loss of wt. during macroscopic sectioning		17,851			20,572
Total wt. of true cortex		36.58 g.			32.89 g.
No. of glomeruli in whole kidney		653,000			676,600

## Maceration method: right kidney

No. of glomeruli in 2 ml.  
macerate, 15 obs.

Wt. of 'quarter' kidney (g.)	Vol. of macerate (ml.)	Mean	S.D. of counts	S.E. of mean	No. of glomeruli in whole kidney
15.45	2,000	175.7	± 17.6	± 4.6	630,000

*Maceration method.* This was applied to the remaining 'quarter' of the right kidney. Fifteen counts were made on 2 ml. volumes of the macerate. The results are given in Table 1. The figure so obtained (630,000) is in satisfactorily close agreement with that derived from the histological method applied to the same kidney (653,000).

## DISCUSSION

The close agreement between the results of two such different methods of estimating the total number of glomeruli in the same dog-kidney leads to the conclusion that both are reasonably accurate when proper precautions are taken. On the other hand there is no comparison between the amounts of time and labour involved in the two techniques. The maceration method requires only 2 or 3 days actual working time spread over a period of several weeks. The histological method requires some 6 months' working time, and there seems to be little chance of this being reduced to a more reasonable length. Inspection of the distribution of glomeruli in the 'quarter' kidney used in the histological computation (Table 1, right kidney), shows that there is considerable variation in the density of the glomerular distribution as one progresses towards the pole of the kidney (C 1, C 2, etc.) and from the lateral border



to the hilum (L 1 H 1; L 2 H 2). A large number of samples of cortex is therefore necessary if a reliable figure for the whole organ is to be obtained. Furthermore, the risk of loss of weight of selected portions of cortex by evaporation of fluid, and hence of too high a value for the number of glomeruli, must be very carefully guarded against. The procedure described with the right kidney seems adequate to prevent this source of error.

The maceration method has been used more frequently in the past chiefly, one feels, because of its comparative simplicity. Inherent in this method, however, is the possibility of considerable error in that there is no check on the number of glomeruli destroyed by the maceration process itself. Direct observation during counting gives some idea of the amount of fragmentation of glomeruli, but this is subjective only. One gains the impression from observing macerates of fresh kidney tissue that fragmentation is sometimes very considerable, so introducing an error which is far from negligible; but with tissue fixed by perfusion *in situ* with 'Susa' fluid the integrity of the glomeruli is much better preserved. At the same time staining of the glomeruli is not necessary. The modification of the maceration method as here described is therefore recommended. The weight of the kidneys after perfusion and fixation *in situ* is of course no indication of their original weight, but if this is needed one kidney can be easily removed and weighed before the other is perfused and fixed.

It would seem, then, that the histological method would be inclined to give too high a figure for the glomerular count because of the evaporative loss of fluid from the small pieces for section, while the maceration method should if anything give too low a figure because of inadvertent destruction of glomeruli. It is true that in our results the figure given by the maceration method is less than that given by the histological one, but the difference is small (3.5 %) thus indicating that the errors can, by proper precautions, be kept within reasonable limits.

Our results confirm the conclusion of Kunkel (1930) that maceration provides a rapid and reliable method for determining the number of glomeruli in a particular kidney.

#### SUMMARY

1. The number of glomeruli in the dog-kidney has been estimated in two ways: by a histological method and by a maceration method.
2. The kidneys were prepared by irrigation *in situ* with physiological saline followed by 'Susa' fluid.
3. The counts by the two methods agreed within 3.5 %.
4. The accuracy and convenience of the two methods is discussed, and the conclusion is reached that the maceration method applied to kidneys fixed by perfusion *in situ* gives a reliable value for the total number of glomeruli.

#### REFERENCES

- BRODIE, T. G. & THACKRAH, M. G. Cited by Brodie, T. G. (1914). Croonian Lecture. A new Conception of the Glomerular Function. *Proc. Roy. Soc. B*, **87**, 571-592.
- KUNKEL, P. A. JR. (1930). The number and size of the glomeruli in the kidney of several mammals. *Johns Hopk. Hosp. Bull.* **47**, 285-291.
- VIMTRUP, B. J. (1928). On the number, shape, structure and surface area of the glomeruli in the kidneys of man and mammals. *Amer. J. Anat.* **41**, 123-151.

# THE INFLUENCE OF CORTISONE ON THE STRUCTURE AND GROWTH OF BONE

By H. A. SISSONS\*

*Institute of Orthopaedics, London*

AND G. J. HADFIELD†

*Professorial Surgical Unit, St Bartholomew's Hospital Medical School, London*

In the course of some experiments on the effects of cortisone administration on the repair of fractures in rabbits (Sissons & Hadfield, 1951), the authors noted, as other workers had done, interference with growth of the experimental animals. This was shown by measurements both of body weight and of the length of long bones, while histological examination of the growing regions of the bones showed marked departure from what was seen in normal control rabbits. The present paper presents in some detail these observations on bone growth.

## METHOD

Young male rabbits about 6 weeks old and male rats about 30 days old were the animals used, and cortisone‡ was administered by daily intramuscular injection. In rabbits, dose levels of 20 mg./kg./day and 10 mg./kg./day were used, while in rats, which in general are more refractory to the effects of cortisone, only the higher dosage of 20 mg./kg./day was used.

In two rabbits (receiving 10 mg./kg./day) and in three rats, measurements of the rate of longitudinal growth of one lower femoral epiphysial plate were made by means of serial radiographs after the implantation of metallic markers into the femoral shaft (Sissons, 1953), and the data so obtained were compared with findings in normal control animals. The remaining groups of cortisone-treated and control animals were killed for histological study at intervals in the period of the experiment, which extended to 24 days for the rabbits and to 31 days for the rats. The growing zones at the lower end of the femur and the upper end of the tibia were studied histologically, longitudinal sections of these bones being prepared after decalcification. A number of staining techniques, including the eosin-azur method, were employed. In this histological material, the thickness of the epiphysial cartilage plates was determined by preparing magnified outline drawings and averaging a number of measurements from each plate. Twenty-one rabbits and thirty-one rats were used in the cortisone groups of the experiment, together with somewhat

\* Part of the experimental work was carried out by one of the authors (H.A.S.) during the tenure of a research fellowship in the Department of Pathology, Northwestern University, Chicago, U.S.A.

† Present address: Department of Surgery, University of Bristol.

‡ Cortisone acetate, suspended in an aqueous medium containing benzyl alcohol 0.9 %, sodium chloride 0.9 %, polyoxyethylene sorbitan mono-oleate 0.4 %, and sodium carboxymethyl cellulose 0.5 %. This substance was provided from a generous gift made jointly to the Medical Research Council and the Nuffield Foundation by Merck and Co. Inc.

larger groups of controls. The experimental rabbits in which longitudinal bone growth was measured were studied for 10 days after the cessation of cortisone administration, and a further group of six animals was used to provide histological material from the growing regions of bones at intervals during this period of recovery.

## RESULTS

### *Rabbits*

Radiographic measurements showed that longitudinal bone growth promptly ceased after the commencement of cortisone administration in animals belonging to both the 10 mg. and 20 mg./kg. groups. The lengths of the measured segment of the



Text-fig. 1. (a) Slab radiograph of lower end of femur in a normal rabbit, showing epiphysal plate and adjacent metaphysal bone trabeculae.  $\times 3.5$ . (b) Slab radiograph of lower end of femur in a rabbit which had received 20 mg./kg. cortisone for 13 days.  $\times 3.5$ .

femur in the experimental animals of the 10 mg./kg. group and the control are shown graphically in Text-fig. 1.

Following cortisone administration, the main structural changes in the growing regions of long bones are narrowing of the epiphysal cartilage plate and progressive reduction in the number of metaphysal bone trabeculae present. These features are evident in radiographs of bones from the cortisone-treated animals (Text-fig. 1), and



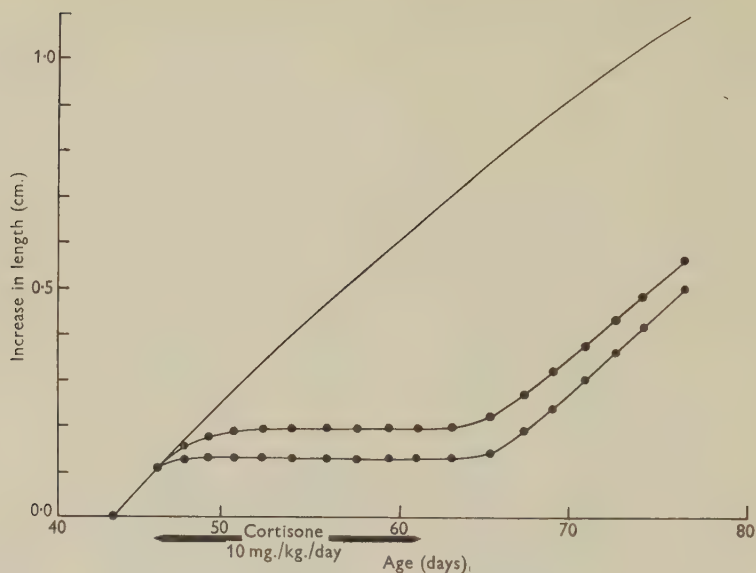
are confirmed by histological study. The thinning of the cartilage plate is present as early as 6 days after the commencement of cortisone administration, and from Table 1 it can be seen that it is as marked then as later in the experiment. The average thickness of the epiphyseal cartilage plates in the experimental animals is  $275\mu$ , compared with an average of  $415\mu$  in the controls. More detailed examination of the abnormal cartilage plates (see Pl. 1, figs. 2, 3) shows that the zone of proliferating cartilage is greatly thinned, while the zones of maturation and calcification are almost completely absent. Metaphyseal blood capillaries are reduced in number, and the normal vascular invasion of the growing surface of the cartilage plate does not occur. The formation and growth of metaphyseal bone trabeculae ceases, and there is progressive reduction in the number and the thickness of these structures, even at some distance from the cartilage plate (see Text-fig. 3). Osteoblasts, which normally form a conspicuous covering layer on the surface of the metaphyseal trabeculae, are

Table 1. *Rabbits. Measurements of epiphyseal plate thickness in longitudinal histological sections*

Control rabbits			Cortisone-treated rabbits			
No.	Day of experiment	Thickness of femoral epiphyseal plate ( $\mu$ )	No.	Dosage (mg./kg./day)	Day of experiment	Thickness of femoral epiphyseal plate ( $\mu$ )
10	4	425	18	20	6	230
14	4	355	13	10	7	225
17	4	450	9	20	9	280
8	5	420	7	20	11	240
5	7	410	26	10	13	365
12	7	335	29	10	13	310
19	8	375	2	20	13	335
24	11	440	11	20	13	245
4	12	375	23	10	15	300
25	14	450	1	20	18	295
22	17	460	21	10	19	270
16	21	470	28	10	22	275
			15	10	24	215

greatly reduced in number, but numerous osteoclasts are present in this situation and appear to be responsible for the progressive disappearance of the bone trabeculae. Although osteoblastic activity is greatly diminished on the surfaces of the metaphyseal trabeculae, osteoblastic bone formation occurs on the inert metaphyseal surface of the cartilage plate and is responsible for the progressive closure of the cartilage plate by a thin—but eventually complete—layer of lamellar bone. Text-fig. 3 (*b*) and Pl. 1, fig. 2, show, for example, when compared with similar areas in a control animal [Text-fig. 3 (*a*) and Pl. 1, fig. 1] the extent to which structural changes have progressed after 11 days of cortisone administration. By 24 days [Text-fig. 3 (*c*) and Pl. 1, fig. 3], more extensive destruction of metaphyseal trabeculae is apparent, and the bony closure of the epiphyseal plate can be seen. Not only is the thin layer of bone visible in histological preparations, but it is also evident in radiographs of bones from the experimental animals—as in Text-fig. 1 (*b*).

All these changes are what might be expected to follow the abrupt cessation of cellular proliferation and longitudinal growth of the cartilage columns of the epi-



Text-fig. 2. Growth curves for lower femoral epiphysial plates in a normal rabbit (—) and in two receiving cortisone (10 mg./kg./day) for 14 days (—●—●—).



Text-fig. 3. Sections through lower ends of femur in control and experimental rabbits. (a) Control (see Pl. 1, fig. 1, for histological appearance of growing region). (b) Cortisone 20 mg./kg. for 11 days (see Pl. 1, fig. 2, for histological appearance of growing region). (c) Cortisone 10 mg./kg. for 24 days (see Pl. 1, fig. 3, for histological appearance of growing region).  $\times 4.5$ .

physial plate, with consequent bony closure of the plate and progressive resorption of metaphysial trabeculae.

Except in one instance, the cells covering the surfaces of any remaining metaphysial bone trabeculae in the animals receiving cortisone, have a normal appearance. The exception was an animal, killed in a moribund condition after 11 days of cortisone administration (20 mg./kg.), where both osteoblasts and osteoclasts had an unusual appearance. Many osteoblasts had lost the normally intense basophilic staining of their cytoplasm, and their nuclei—normally large and vesicular—were small and pyknotic. Osteoclasts appeared to be less severely affected, but the granularity of their cytoplasm was increased and their nuclei stained more darkly than usual.

In some of the experimental animals of the 10 mg./kg. group, cortisone administration was discontinued after 14 days. The results are shown in Text-fig. 2, where it can be seen that longitudinal bone growth was resumed—after an interval of about 5 days—at an approximately normal rate of 0.33 mm./day. Three animals killed for histological study at 1, 5 and 7 days after the cessation of cortisone administration failed to show any evidence of regrowth, and still possessed thinned epiphysial plates. However, one animal killed at 10 days showed an epiphysial plate of normal thickness ( $475\mu$ ), together with a zone of newly developed bone trabeculae on its metaphysial aspect approximately 0.7 mm. wide (Pl. 1, fig. 4), corresponding to 2 days of longitudinal growth at the normal rate. On its metaphysial surface this zone of regrowth is limited by a thin layer of bone and cartilage which is the remains of the inert surface layer of the 'arrested' plate, now displaced from the resting cartilage of the 'reactivated' plate by the zone of new tissue formed since the cessation of cortisone administration.

### *Rats*

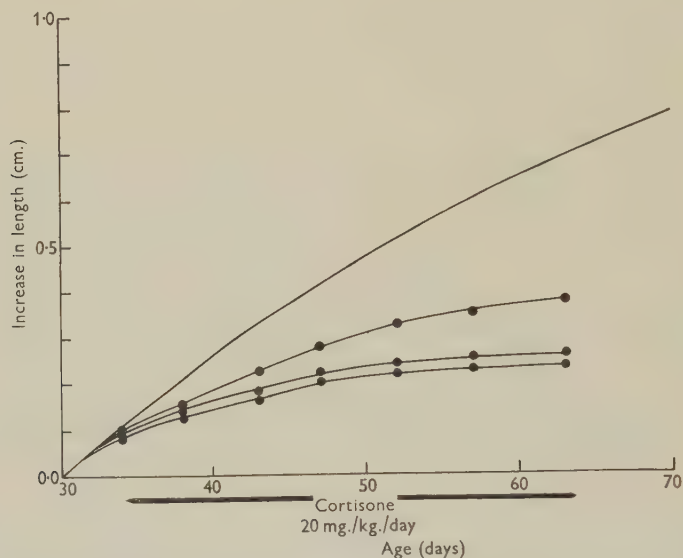
With rats, the findings were in general similar to those described with rabbits, but the growth retardation was not complete and the histological changes were not so marked. Text-fig. 4 records graphically the values for bone growth in the three animals receiving 20 mg./kg./day cortisone, compared with a growth curve for normal rats\* of the same age. It can be seen that a diminished, but measurable, amount of bone growth occurred throughout the experiment.

Concerning structural changes in the growing regions of long bones following cortisone administration, some outstanding differences are found between two groups of the experimental animals. The first and larger of these groups was part of an experiment carried out in London, while the second—consisting only of six animals—was studied in Chicago. Each group consisted of males from colonies of Wistar type rats, and each received the same amount of an identical cortisone preparation. Weight records showed a similar degree of interference with general growth in the two groups, and X-ray measurements indicated a similar retardation of bone growth. Histological studies of bones showed a similar degree of thinning of the epiphysial plates in the two groups (see Table 2), but while the first series of animals showed the same progressive reduction in the number of metaphysial trabeculae already

\* The individual figures for these normal animals are given in a previous publication (Sissons, 1953) where they are referred to as rats 1, 2 and 3 of Table 1.



described for rabbits, the second series showed a uniform and surprisingly contrasting increase in the density of the same metaphysial bone in a zone approximately 2–3 mm. wide adjacent to the epiphysial plate. In each group there was some degree of closure of the epiphysial plate by an incomplete layer of lamellar bone. Over the whole period of the experiment the average thickness of the epiphysial cartilage plates in the experimental animals is  $170\mu$  compared with an



Text-fig. 4. Growth curves for lower femoral epiphysial plates in normal rats (—), and in three rats receiving cortisone (20 mg./kg./day) (—●—●—).

Table 2. *Rats. Average measurements of epiphysial plate thickness in longitudinal histological sections*

Period of experiment (days)	No. of experimental animals	Plate thickness ( $\mu$ )	Plate thickness ( $\mu$ ) of corresponding controls
5	3	195	270
6–10	7	150	280
11–15	8	160	280
16–25	4	185	265
26–31	9	180	300

average of  $285\mu$  in the controls. The contrasting appearance of the metaphysial bone in the two groups is shown in Text-fig. 5, and in Pl. 1, figs. 6 and 7. Pl. 1, fig. 6, shows the sparsity of metaphysial bone in an animal of the first series after 26 days of cortisone administration; Pl. 1, fig. 7, shows the network of dense bone trabeculae with prominent cartilaginous inclusions on the metaphysial side of the epiphysial plate in an animal from the second series after a period of cortisone administration of approximately the same duration.

## DISCUSSION

The effect of cortisone in inhibiting the growth of a developing animal was first described by Wells & Kendall (1940), on the basis of experiments with rats. Since then its inhibitory effects have been extended to a wide variety of proliferating tissues, including the skin (Baker, Ingle, Li & Evans, 1948), granulation tissue of healing wounds (Howes, Plotz, Blunt & Ragan, 1950), fracture callus (Blunt, Plotz, Lattes, Howes, Meyer & Ragan, 1950; Sissons & Hadfield, 1951), and the tissues of the developing chick embryo (Karnofsky, Ridgway & Patterson, 1951). Experiments involving local painting of cortisone to the skin (Baker & Whitaker, 1948;



Text-fig. 5. Sections through lower ends of femur in control and experimental rats. (a) Control (see Pl. 1, fig. 5, for histological appearance of growing region). (b) 1st cortisone series (cortisone 20 mg./kg. for 28 days), see Pl. 1, fig. 6, for histological appearance of growing region in a comparable animal of this series. (c) 2nd cortisone series (cortisone 20 mg./kg. for 31 days), see Pl. 1, fig. 7, for histological appearance of thickened metaphysal trabeculae.  $\times 8$ .

Castor & Baker, 1950), and on the effect of cortisone on explanted tissues (Bullough, 1952) and tissue cultures (Barber & Delaunay, 1951; Ruskin, Pomerat & Ruskin, 1951) have demonstrated a direct effect of the hormone on the cells of the proliferating tissue, although Steen (1951) and Barski & Brion (1952) have shown that the necessary concentration of cortisone is greatly in excess of the effective level in the intact animal.

Follis (1951 *a, b*) has briefly described the histological changes in the growing bones of animals receiving cortisone. With mice, guinea-pigs and rabbits he found that the proliferation of epiphysal cartilage was inhibited. With rats, this was accompanied by retardation of the normal osteolytic sequences in the metaphysal bone, with consequent increased bone density and persistence of abnormally large amounts of

cartilage matrix in the bone trabeculae in this region. Cavallero, Bertazzoli, Rossi & Sala (1951) have also very briefly noted histological changes, consisting of narrowing of the epiphysial plate, shortening in irregularity of the columns of cartilage cells, and reduction in the number of metaphysial trabeculae, in the bones of rats receiving cortisone.

In the present experiments, the attempt has been made, both in rabbits and rats, to correlate the histological changes produced by cortisone in the growing regions of long bones with the growth rates of the same bones, determined by serial radiographic measurements. In rabbits, complete cessation of longitudinal bone growth is easily produced and results in prompt narrowing of the epiphysial plate, disappearance of hypertrophic cartilage cells, and cessation of proliferation of the cells of the cartilage columns. Persistence of osteoclastic resorption of bone trabeculae in the metaphysial region leads to progressive disappearance of these structures, and there is eventual closure of the surface of the inert cartilage plate by a layer of bone. Doses of cortisone which completely arrested bone growth in the rabbit had less severe effects in rats, but the retardation of longitudinal growth in this species was accompanied by similar histological changes in the epiphysial cartilage plates. In contrast to the experiments of Follis, it was found that in rats, a dose level of cortisone which produced severe interference with epiphysial growth did not invariably produce increased density of bone in the metaphysial region. The type of response is thus not solely a question of species difference, but must be determined by factors which were unknowingly varied between the two rat groups described. In which the majority of animals responded in a similar way to the rabbit, the guinea-pig and the mouse. The structure of the metaphysial bone trabeculae is determined by a balance between opposed osteoblastic and osteoclastic activities, and each of the observed effects of cortisone on these structures can be explained by a changed balance, favouring one or other of the two cellular processes. In the rats of the second series in the present experiments, the width of the zone of dense metaphysial bone is approximately the same as the amount of longitudinal bone growth during the period of cortisone administration, indicating that it is chiefly the bone formed during this period which fails to undergo the normal osteoclastic resorption.

It must be remembered that each of the two patterns of response in the rat is a non-specific one. The association of cessation or retardation of growth with resorption of metaphysial trabeculae, is found, for instance, in inanition, although it is clear that in animals treated with cortisone the changes are not merely the result of a diminished food intake. Both rabbits and rats receiving cortisone continued to eat relatively normal amounts of food despite their failure of growth or even weight loss, and in the experiments of Follis (1951*a*) a degree of dietary restriction which produced weight curves similar to those of animals receiving cortisone failed to produce such severe retardation of epiphysial growth. Particularly in the rabbit, the administration of cortisone can rapidly bring about far more acute changes in the growing regions of bones than even complete starvation. Not only inanition, but the arrest of bone growth which follows hypophysectomy (Becks, Kibrick, Marx & Evans, 1941), results in the same type of structural change noted in these cortisone-treated animals. The increased density of metaphysial bone, too, although not seen



in inanition or following hypophysectomy, is found following administration of oestrogens in rats (Day & Follis, 1941; Budy, Urist & McLean, 1952), where it is associated with retardation of bone growth.

#### SUMMARY

1. In rabbits and rats, the administration of cortisone at a dose level of 20 mg./kg./day results in retardation of the growth of long bones, and produces structural changes in the epiphysial plates and metaphysial trabeculae.

2. In rabbits the dosage produces complete arrest of epiphysial bone growth, although this is resumed within a few days of the cessation of cortisone administration.

3. In rats the retardation of bone growth and the structural changes in the growing zones are less pronounced.

4. The increased density of metaphysial bone, described in the rat by Follis, is not an invariable result, in this species, of doses of cortisone sufficient to produce marked retardation of longitudinal bone growth.

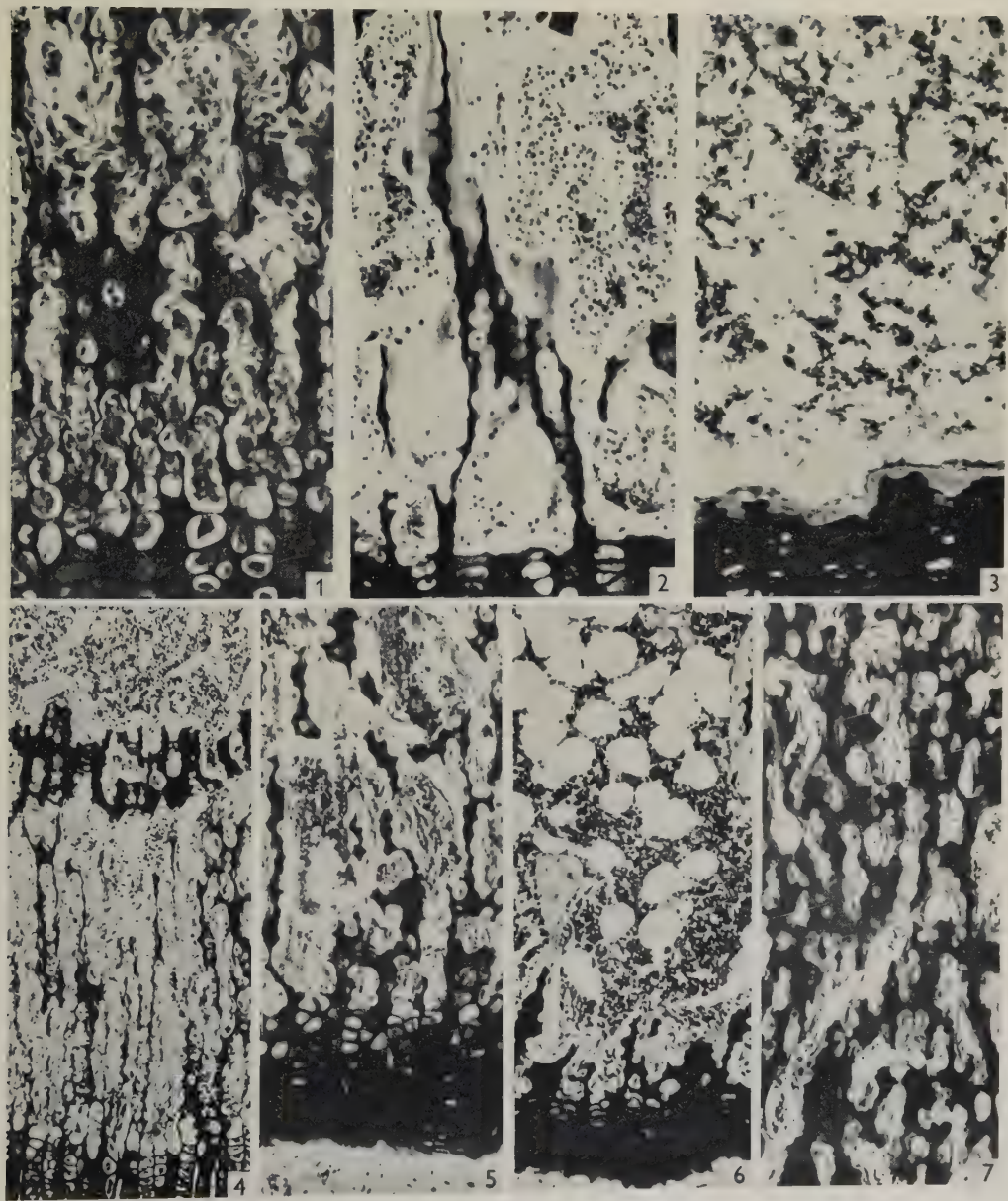
#### REFERENCES

- BAKER, B. L., INGLE, D. J., LI, C. H. & EVANS, H. M. (1948). Growth inhibition in the skin induced by parenteral administration of adrenocorticotropin. *Anat. Rec.* **102**, 313-331.
- BAKER, B. L. & WHITAKER, W. L. (1948). Growing inhibition in the skin following direct application of adrenal cortical preparations. *Anat. Rec.* **102**, 333-347.
- BARBER, M. & DELAUNAY, A. (1951). Effet du plasma prélevé chez des cobayes traités par la cortisone sur des cultures in vitro de fibroblastes et de macrophages. *Ann. Inst. Pasteur*, **81**, 193-205.
- BARSKI, G. & BRION, G. de (1952). Culture prolongée du tissu conjonctif et épiphysial des mammi-fères en présence de cortisone et de désoxycorticosterone. *Ann. Inst. Pasteur*, **82**, 563-577.
- BECKS, H., KIBRICK, E. A., MARX, W. & EVANS, H. M. (1941). The early effect of hypophysectomy and of immediate growth hormone therapy on endochondral bone formation. *Growth*, **5**, 449-456.
- BLUNT, J. W., PLOTZ, C. M., LATTES, R., HOWES, E. L., MEYER, K. & RAGAN, C. (1950). Effect of cortisone on experimental fractures in the rabbit. *Proc. Soc. exp. Biol., N.Y.*, **73**, 678-681.
- BUDY, A. M., URIST, M. R. & MCLEAN, F. C. (1952). The effect of estrogens on the growth apparatus of the bones of immature rats. *Amer. J. Path.* **28**, 1143-1167.
- BULLOUGH, W. S. (1952). Stress and epidermal mitotic activity. 1. The effects of the adrenal hormones. *J. Endocrin.* **8**, 365-376.
- CASTOR, C. W. & BAKER, B. L. (1950). The local action of adreno-cortical steroids on epidermis and connective tissue of the skin. *Endocrinology*, **47**, 234-241.
- CAVALLERO, C., BERTAZZOLI, C., ROSSI, L. & SALA, G. (1951). Studio sperimentale sugli effetti del cortisone. II Effetti morfologici generali sul ratto normale. *Sperimentale*, **101**, 209-225.
- DAY, H. G. & FOLLIS, R. H. JNR. (1941). Skeletal changes in rats receiving estradol benzoate as indicated by histological studies and determinations of bone ash, serum calcium and phosphatase. *Endocrinology*, **28**, 83-93.
- FOLLIS, R. H. (1951a). Effect of cortisone on growing bones of the rat. *Proc. Soc. exp. Biol.* **76**, 722-724.
- FOLLIS, R. H. (1951b). Non-effect of cortisone on growing bones of mice, guinea-pigs, and rabbits. *Proc. Soc. exp. Biol.* **78**, 723-724.
- HOWES, E. L., PLOTZ, C. M., BLUNT, J. W. & RAGAN, C. (1950). Retardation of wound healing by cortisone. *Surgery*, **28**, 177-181.
- KARNOFSKY, D. A., RIDGWAY, L. P. & PATTERSON, P. A. (1951). Growth-inhibiting effect of cortisone acetate on the chick embryo. *Endocrinology*, **48**, 596-616.

- RUSKIN, B., POMERAT, C. M. & RUSKIN, A. (1951). Toxicity of various cortisone preparations on embryonic chick heart, spleen and spinal cord in tissue culture. *Texas Rep. Biol. Med.* **9**, 786-795.
- SISSONS, H. A. (1953). Experimental determination of rate of longitudinal bone growth. *J. Anat., Lond.*, **87**, 228-236.
- SISSONS, H. A. & HADFIELD, G. J. (1951). The influence of cortisone on the repair of experimental fractures in the rabbit. *Brit. J. Surg.* **39**, 172-178.
- STEEN, A. S. (1951). Effect of cortisone on tissue cultures. *Brit. J. Ophth.* **35**, 741-749.
- WELLS, B. B. & KENDALL, E. C. (1940). The influence of corticosterone and C<sub>17</sub> hydroxydehydrocorticosterone (compound E) on somatic growth. *Proc. Mayo Clin.* **15**, 324-328.

## EXPLANATION OF PLATE

- Fig. 1. Photomicrograph showing the hypertrophic cartilage of the femoral epiphysial plate and the adjacent metaphysial bone trabeculae in a normal rabbit.  $\times 128$ .
- Fig. 2. Rabbit. Cortisone 20 mg./kg. for 11 days. Histological appearance of same region, showing the absence of hypertrophic cartilage and the diminished number of metaphysial trabeculae.  $\times 128$ .
- Fig. 3. Rabbit. Cortisone 10 mg./kg. for 24 days. Histological appearance of same region; the proximal surface of the epiphysial plate is covered with a thin layer of bone.  $\times 128$ .
- Fig. 4. Rabbit. Cortisone 10 mg./kg. for 14 days. Killed 10 days after cessation of cortisone administration. Note the zone of newly developed bone separating the surface layer of the 'arrested' plate (above) from the resting cartilage of the 'reactivated' plate (below).  $\times 64$ .
- Fig. 5. Histological appearance of the growing region of the femoral epiphysial plate in a normal rat.  $\times 112$ .
- Fig. 6. Rat (1st series). Cortisone 20 mg./kg. for 26 days. Histological appearance of the same region, showing the thinned plate and the diminished number of metaphysial bone trabeculae.  $\times 112$ .
- Fig. 7. Rat (2nd series). Cortisone 20 mg./kg. for 31 days. Histological appearance of the abnormally thick metaphysial bone trabeculae containing a conspicuous network of unresorbed epiphysial cartilage.  $\times 112$ .



SISSONS AND HADFIELD—CORTISONE ADMINISTRATION ON STRUCTURE AND GROWTH OF BONE





# THE EFFECTS OF PARTIAL OR COMPLETE EXCISION OF THE EPIPHYSEAL CARTILAGE OF THE RABBIT

By P. A. RING

*Charing Cross Hospital Medical School, London*

The relation of the epiphyseal cartilage of long bones to their growth, and the effects of removal of these cartilages, has been extensively studied. From time to time regeneration of the growth cartilage has been observed following excision of this area (Selye, 1934; Lacroix, 1951), but considerable evidence has accumulated to suggest that restoration of full bone length is rare, both in the experimental animal and in man. Even when regeneration has not occurred, however, increase in length of the affected bone has been described (Hellstadius, 1947). This observation casts some doubt upon the role of the epiphyseal cartilage in bone growth, and the present series of experiments was undertaken to shed more light upon this problem and to define the functions of the different areas.

In the young rabbit the distal end of the ulna lends itself readily to this work. It is subcutaneous, and grows rapidly; observations during this study suggest it contributes some 85 % of the total postnatal growth of this bone. The epiphyseal cartilage in the young animal is broad, and this permits selective excision of different areas to be performed. The close association of the ulna with the radius renders post-operative splinting unnecessary, although this same relationship complicates the evaluation of the results achieved.

## METHOD

Rabbits aged from 2 to 5 weeks were used. Each animal was anaesthetized with ether, and the forelimb approached by a longitudinal incision. The distal end of the ulna was identified, and exposed for most of its circumference by freeing and retracting the adjacent tendons. One of three distinct procedures was then adopted.

(a) Complete excision of the epiphyseal cartilage by transverse cuts passing through the immediately adjacent metaphyseal and epiphyseal bone (Text-fig. 1A).

(b) Excision of the reserve zone by transverse cuts through the centre of the cartilage and the epiphyseal bone (Text-fig. 1B).

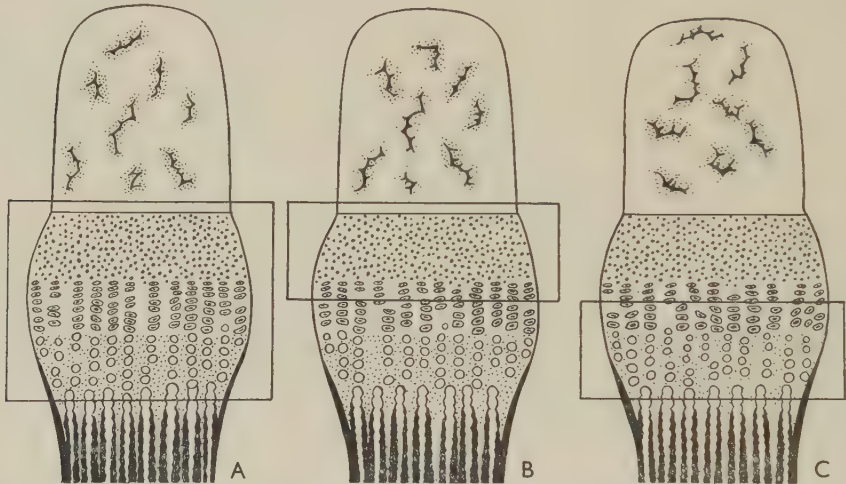
(c) Excision of the area containing cartilage columns and the surrounding perichondrial ring by transverse cuts through the centre of the cartilage and the metaphyseal bone (Text-fig. 1C).

In each case the portion of bone and cartilage removed was sectioned serially to verify the extent of the lesion.

Into the shaft of the ulna on both operated and control sides was inserted a small piece of lead shot to act as a radiological marker. Each animal was radiographed after the operation, and thereafter at weekly, and later fortnightly, intervals. Animals were weaned from the mother at 6 weeks, and were subsequently kept in a large wire run. They received a normal mixed diet supplemented by pellet feeding.

At varying periods up to 6 months biopsies were performed. The animals were killed with nembutal, and the limbs radiographed. The bones of the forelimb were fixed in formol saline or Susa, and later decalcified. Following decalcification the distal ends of the radius and ulna were removed and sectioned serially.

All radiographs were taken with the animal in the prone position, each limb flexed at the elbow, and the paw outstretched giving a standard dorso-ventral view of the distal part of the limb and a lateral view of the proximal part. The tube-film distance was constant at 100 cm. A number of measurements made upon the isolated bones and upon teleradiographs showed that the error in the method due to magnification was less than 0.5 mm. and was equal on both sides. Each ulna was measured from its most proximal point to the centre of the distal articular surface. The distance from the centre of the marker to each end was also noted. In



Text-fig. 1. The distal epiphyseal cartilage of the ulna of the rabbit, indicating extent of complete excision (A), excision of distal segment (B) and of proximal segment (C).

an ulna markedly curved as a result of more rapid growth of the radius, the total length thus recorded was less than the sum of the subsidiary measurements. It was also discovered that the lead marker failed to represent a fixed point in the shaft under two conditions.

- (a) When it was placed within the medullary cavity at the original operation.
- (b) When it came to lie within the medulla as a result of increased size of the bone as a whole.

Control observations with steel pins, however, showed that errors occurring in this way were small.

#### OBSERVATIONS

##### (a) *Total excision*

Of the animals subjected to complete excision of the epiphyseal cartilage and the adjacent bone, ten were available for continued radiological study, and of these histological sections were produced from seven. A further five animals which survived for a month or less provided material for histological examination alone.



Measurements upon the radiographs of eight of the ten animals examined showed a marked shortening of the ulna at the end of the period of study, whilst two (nos. 164 and 165) showed no significant disturbance of growth (Table 1). During the first 3 weeks after operation the ulna on the operated side increased in length to an extent comparable with the control. After this period further increase in length was unusual (Table 2), and the radiographs commonly demonstrated bony union between epiphysis and diaphysis. The arrest of growth was more marked when the total length of the bone was analysed by assessing the contribution from each end (Table 2).

From the radiographs alone it was difficult to determine or deduce the fate of the epiphyseal cartilage. The radiotranslucent area between metaphysis and epiphysis became smaller, and was often obscured by opaque bands suggesting union between the two fragments. Commonly, however, a bony spur was seen on the ulnar aspect of the radius, and either the proximal, or the distal fragment of the ulna, or both, appeared to articulate with it.

Histological examination showed that in the first week the gap between the epiphysis and diaphysis was filled with richly vascular and cellular connective tissue. From the cut bone ends clumps of osteoblasts were seen growing into the connective tissue. After 11 days, cartilage cells were seen in small numbers, and by 18 days the gap was occupied mainly by cartilage cells. After this period all but two of the animals showed a more or less completely organized epiphyseal plate usually associated with a small spur projecting from the radius.

The histological and final radiological findings are considered in detail below.

#### *R. 185. Survival 6 days*

The gap between diaphysis and epiphysis is filled by a richly vascular and cellular connective tissue. The cut ends of the bone are regular and against each surface are clumps of osteoblasts. The edge of the radius against the scar shows a collection of proliferating cartilage cells.

#### *R. 115. Survival 9 days*

The cut ends are markedly irregular. From the more prominent lamellae long columns of osteoblasts extend into the scar tissue until they merge indistinctly into the longitudinally running fibroblasts. Many large vessels are seen within the connective tissue (Pl. 1, fig. 5).

#### *R. 186. Survival 11 days*

The scar comprises a richly cellular fibrous tissue containing long columns of osteoblasts. Cartilage cells are seen in irregular clumps mainly on the epiphyseal side of the lesion.

#### *R. 116. Survival 16 days*

A considerable amount of cartilage is visible within the connective tissue scar. The cartilage cells are grouped in longitudinal columns between the bone ends and around the bony spur which has developed on the ulnar side of the radius. Few osteoblasts can be seen.

#### *R. 187. Survival 18 days*

The gap between epiphyseal and diaphyseal bone is occupied mainly by cartilage cells. These cells show none of the organized arrangement of an epiphyseal cartilage.

*R. 166.* Survival 4 weeks. Shortening 8.5 mm.

The radiographs show an outward curvature of the radius and a valgus deformity of the paw. The distal end of the ulna is also curved outwards. There appears to be bony union between epiphysis and diaphysis.

Histologically the epiphysis and diaphysis are united by bone. The operation area is marked by a peripheral ring of cartilage.

*R. 164.* Survival 5 weeks. No shortening

The radiographs show a slight outward curvature of the radius which has developed on its ulnar aspect a bony spur which separates the epiphyseal fragment of the ulna from the ulnar shaft. The films show no evidence of bony union (Pl. 1, fig. 1).

Serial sections show that the epiphysis is attached to the distal aspect of the radial spur by a mass of cartilage. The cartilage cells are arranged on the proximal aspect in columns which differ from an epiphyseal cartilage only in their irregularity. This cartilaginous area is nowhere continuous with the epiphyseal cartilage of the radius. The bony spur from the radius shows the structure characteristic of the metaphysis of any long bone (Pl. 1, figs. 6, 7). The diaphysis of the ulna is regular and at its tip appears encapsulated by fibrous tissue.

*R. 165.* Survival 5 weeks. No shortening

The radiographs show no shortening of the ulna and no curvature of the forearm bones. The shaft of the ulna is drawn out to a point. It is separated from the epiphysis by a radio-translucent area into which a small radial spur projects.

The histological sections show a wide band of cartilage between the radial spur and the distal fragment of ulna. This cartilage shows well-marked columns on both proximal and distal aspects (Pl. 2, fig. 8). The ulnar diaphysis terminates freely and is capped by connective tissue.

*R. 167.* Survival 5 weeks. Shortening 10 mm.

Bony union between epiphysis and diaphysis is apparent on both radiographs and sections. The operation area is surrounded by small islands of cartilage.

*R. 161.* Survival 6 weeks. Shortening 8.5 mm.

The radiographs show marked curvature of the radius which is associated with clubbing of its distal end. The epiphysis of the radius appears fused with that of the ulna and an indistinct curved epiphyseal line can be traced common to both bones (Pl. 1, fig. 2).

The histological sections show that the radial epiphyseal cartilage is very broad and extends for some distance on the ulnar aspect of the bone. The ulnar epiphyseal cartilage is narrow and markedly irregular. On its distal aspect there are numerous cartilage cell columns; proximally lies a mass of unorganized cartilage continuous at one point with the epiphyseal cartilage of the radius.

*R. 163.* Survival 8 weeks. Shortening 15 mm.

There is considerable outward curvature of the radius associated with a valgus deformity of the paw. The metaphysis of the ulna is expanded and is united to the epiphysis by bone.

*R. 162.* Survival 9 weeks. Shortening 16.5 mm.

The radiographs show bony union has occurred between the epiphysis and diaphysis and there is marked outward deviation of the forefoot. From the radius a small spur projects towards a cavity on the radial aspect of the ulna.

Examination of the serial sections confirms the bony union apparent in the radiographs. Against the bony spur of the radius are islands of cartilage with cell columns irregularly arranged within their substance. These islands are continuous in some sections with a well-marked epiphyseal plate, completely surrounded by bone, in the centre of the ulna (Pl. 2, fig. 9).

*R. 83. Survival 10 weeks. Shortening 17 mm.*

The radiographs show a marked shortening of the radius and ulna and an outward curvature of the paw. An irregular thin epiphyseal line is apparent in the ulna (Pl. 2, fig. 10).

Histologically the operated area is occupied by new bone containing in its centre a thin plate of epiphyseal cartilage, the cell columns of which are very short.

*R. 66. Survival 11 weeks. Shortening 11.5 mm.*

The radiographs show a valgus deformity of the paw associated with a tilting of the distal epiphysis of the radius. There is no epiphyseal line visible, and firm bony union has occurred between epiphysis and metaphysis.

*R. 105. Survival 11 weeks. Shortening 22 mm.*

The radiographs show a marked valgus deformity of the paw. The diaphysis and epiphysis of the ulna are united by a narrow band of bone.

Table 1. *Increase in length of ulna following complete excision of epiphyseal cartilage*

No.	Age at operation (days)	Post-operative survival (weeks)	Growth in first 3 weeks		Total post-operative growth	
			Operated (mm.)	Control (mm.)	Operated (mm.)	Control (mm.)
66	31	11	2.5	2.5	4.5	16
83	27	10	4	4.5	7.5	24.5
105	31	11	7	10.5	12	34
161	14	6	10.5	15.5	17.5	26
162	21	9	6.5	13.5	8.5	25
163	21	8	11	18	13	28
164	28	5	5	5.5	14	14
165	28	5	12	13.5	17	17
166	28	4	3.5	12	3.5	12
167	28	5	6	13	7	17

Table 2. *Complete excision of ulnar epiphyseal cartilage. Growth between period 3 weeks after operation and biopsy*

No.	Operated		Control	
	Total (mm.)	Distal end (mm.)	Total (mm.)	Distal end (mm.)
66	2	0	13.5	*
83	3.5	*	20	*
105	5	0	23.5	19
161	7	3	10.5	7
162	2	0.5	11.5	9
163	2	0	10	7
164	9	7.5	9.5	7
165	5	3.5	3.5	3
166	0	0	0	0
167	1	0	4	2

\* Not measured.

*(b) Excision of distal segment*

In this group six animals were available for serial radiographs, and of these, four for histology. A further nine animals were sacrificed for histological examination alone of the earlier stages of the lesion.



Measurements upon the radiographs showed considerable shortening at the end of the period of study, the difference in length between the two bones varying between 1 and 21 mm. During the first few weeks increase in length of the ulna was almost normal, but after this period the ulna grew less rapidly than the radius, and an outward curvature of the radius usually resulted. Many of the later radiographs appeared to show bony union between the two ulnar fragments; others showed a rounding of the metaphysis of the ulna, and often a clubbing of this bone.

Examination of the histological sections showed that in the first few days the operation area was filled with blood clot which later became replaced by a vascular fibrous scar. The cartilage columns appeared to shorten and become irregular during the second post-operative week. In some animals no columns remained after 14 days, in others the columns were irregular, with no living cells. Where these columns were capped by a limiting band of fibrous tissue they appeared to represent structures persisting from the pre-operative period (R. 208). In other animals (R. 207 and R. 212) the columns were surmounted by a reserve zone of immature cartilage cells proximal to the operation site. In these animals complete degeneration of the original cartilage columns appeared to be followed by regeneration from the metaphyseal area. Subsequent events in the area of excision appeared variable; either the scar tissue was replaced by bone, or, in two cases, a thin band of epiphyseal cartilage was seen and was associated with relatively normal growth.

*R. 209. Survival 1 week*

The area of the excision is occupied in part by fibrous tissue, with large vessels within it, and in part by small, irregularly disposed, cartilage cells. The proximal, intact, part of the epiphyseal cartilage has become rounded off, and is covered distally by blood clot. Arising from the radial aspect of the metaphysis are long columns of new bone, lacking the basophilic centre characteristic of bone surrounding calcified cartilage.

*R. 228. Survival 1 week*

The sections show the remaining epiphyseal cartilage to consist of long columns of cells with small, pyknotic nuclei. The operation area is filled with blood clot (Pl. 2, fig. 11).

*R. 217. Survival 11 days*

The bulk of the scar is occupied by richly vascular fibrous tissue. The cartilage columns left intact after the operation are now visible only as small clumps against the metaphysis.

*R. 210. Survival 12 days*

No cartilage columns are visible. The scar is occupied by fibrous tissue within which are grouped small islands of cartilage.

*R. 215. Survival 2 weeks*

Short cartilage columns are visible in this specimen. On the ulnar aspect of the radius lies a large mass of cartilage which extends for some distance between the epiphysis and metaphysis of the ulna.

*R. 212. Survival 2 weeks*

Cartilage columns are seen in the centre of this specimen. Distal to these, the operated area contains a dense fibrous tissue.

*R. 214. Survival 2 weeks*

No cartilage columns are visible. The bulk of the scar consists of a dense fibrous tissue into which some cartilage cells appear to extend from the radius.

**R. 208. Survival 2 weeks**

Cartilage columns persist only in the centre of this specimen where the cells are degenerate and the matrix calcified. The columns are capped by a limiting band of fibrous tissue. Distal to this lie a number of free cartilage cells within a connective tissue framework.

**R. 207. Survival 2 weeks**

The line indicating the excised area is clearly seen. Proximal to this lie a group of apparently normal cartilage columns, distal to the line is a number of young cartilage cells (Pl. 2, fig. 12).

**R. 216. Survival 6 weeks. Shortening 9 mm.**

The radiographs show an outward curvature of the radius and ulna. The epiphysis of the ulna appears to have united with the metaphysis.

The sections confirm bony union between the epiphysis and metaphysis. Around the edges of this bony bridge are many cartilage cells, some with a vesicular cytoplasm.

**R. 213. Survival 8 weeks. Shortening 5 mm.**

The radius and ulna are curved outwards. The operation site is occupied by a V-shaped radiotranslucent area.

**R. 114. Survival 9 weeks. Shortening 1 mm.**

The radiographs show the ulnar metaphysis has become rounded. There is a narrow epiphyseal line, and the epiphysis itself is considerably larger and longer than its control. There is a small spur arising from the radius.

The sections show that the distal aspect of the curved metaphysis is occupied by a narrow band of epiphyseal cartilage comprising mainly cartilage columns. Distal to this is an irregular, broad, cartilaginous area separated from the columns by a small bony plate.

**R. 110. Survival 11 weeks. Shortening 6 mm.**

The radiographs show an irregular V-shaped gap between epiphysis and diaphysis. There is a bony spur on the outer aspect of the radius.

Examination of the sections shows the metaphysis of the ulna capped with a narrow band of epiphyseal cartilage, with regular cartilage columns and a short reserve zone.

**R. 112. Survival 15 weeks. Shortening 16 mm.**

The radiographs show an expanded metaphyseal region joined to the epiphysis by a thin band of bone. There is no evidence of an epiphyseal cartilage.

The sections show the shaft and epiphysis of the ulna united by a thin bar of cancellous bone. There is no epiphyseal cartilage visible in or around the ulna.

**R. 113. Survival 30 weeks. Shortening 21 mm.**

The distal end of the radius is curved outwards and is clubbed. The epiphysis appears to have united with the shaft of the ulna with a considerable angular displacement. The metaphyseal region of the ulna is very much wider than normal and is relatively radio-translucent (Pl. 1, fig. 3).

Table 3. *Increase in length of ulna following excision of distal segment of epiphyseal cartilage*

No.	Age at operation (days)	Post-operative survival (weeks)	Growth in first 3 weeks		Total post-operative growth	
			Operated (mm.)	Control (mm.)	Operated (mm.)	Control (mm.)
110	21	11	13	16	23	29
112	21	15	14.5	16.5	18.5	34.5
113	21	30	15	16	22	43
114	21	9	16	16	21.5	22.5
213	29	8	6	8	12	17
216	29	6	2.5	6.5	3	12

*(c) Excision of proximal segment*

Serial radiographs were available of seven animals which survived for periods up to 22 weeks. Five of these animals were examined histologically, together with seven animals surviving for less than 1 month.

Measurements of the radiographs showed that post-operative growth in all cases was considerable; in six of the animals the shortening was 6 mm. or less—the other animal showed 17 mm. of shortening. The serial radiographs of all the animals showed an area between epiphysis and diaphysis which remained radio-translucent and in most there was a cloudiness on the diaphyseal side suggesting an actively functioning metaphysis. Some of the radiographs showed slight radial curvature; this was in general proportional to the amount of shortening of the ulna.

Examination of the sections showed that in the early post-operative days the gap was filled with a haematoma. The cut edges of the cartilage remained as a sharply defined area, but on the metaphyseal side the bony margin showed an irregularity which became more marked after the first few days. Columns of osteoblasts passed into the haematoma and occasional cartilage cells were seen. Four weeks after the operation the epiphyseal cartilage appeared as a wide area with many mature and degenerating cartilage cells surrounded by a calcified ground substance. In these later specimens the cut surface of the cartilage was often still apparent, suggesting that this proliferating and degenerating cartilage arose from the diaphyseal side, rather than from degeneration of the cartilage cells left after the original operation. Reformation of the perichondrial osseous ring was apparent in the later specimens.

*R. 202. Survival 3 days*

On the distal aspect of the epiphyseal cartilage the undisturbed reserve zone is visible. The area of operation is filled with blood clot.

*R. 205. Survival 3 days*

On the distal aspect of the epiphyseal cartilage the reserve area and the cartilage columns are visible. (The area of excision in this specimen included only a very narrow band of cartilage most of which was calcified.) The operation area is filled by blood clot.

*R. 200. Survival 3 days*

The area of the operation is filled with blood clot.

*R. 204. Survival 6 days*

The line of the incision through the epiphyseal cartilage is clearly marked. Proximally lies a blood clot invaded by a few connective tissue cells.

*R. 201. Survival 6 days*

The distal, undisturbed cartilage shows on its proximal aspect some irregular cartilage columns. The line of the excision appears irregular and the operated area contains a richly vascular connective tissue into which numerous osteoblasts extend. The osteoblasts arise from the metaphyseal region and are associated with the formation of thin columns of new bone within the scar (Pl. 2, fig. 13).

*R. 203. Survival 7 days*

A few columns are visible within the surviving cartilage. The scar itself contains many blood vessels and osteoblasts and a little new bone arising on the metaphyseal side.



*R. 225. Survival 2 weeks*

The area of operation is occupied almost entirely by calcified cartilage cells which are in the main degenerate and are surrounded by a calcified interstitial substance. These cells are continuous distally with the area of epiphyseal cartilage which was left undisturbed. Proximally they are separated from the metaphysis by fibrous tissue (Pl. 2, fig. 14).

*R. 232. Survival 4 weeks. Shortening 3 mm.*

The radiographs show some outward curvature of the forelimb. The gap between epiphysis and metaphysis is mainly radio-translucent but there is an opacity on the radial side.

The sections show a wide band of cartilage passing between the two fragments on the ulnar side; the radial side is filled with connective tissue and a little new bone. The continuous band of cartilage shows a few short columns on the metaphyseal side (Pl. 2, fig. 15).

*R. 230. Survival 5 weeks. Shortening 5 mm.*

The final radiograph shows some outward curvature of the ulna. There is a small spur arising from the radius with which part of the ulnar epiphysis appears to have fused. The gap between epiphysis and metaphysis of the ulna is elsewhere radio-translucent.

The area of the epiphyseal cartilage is divided by a gap which appears to represent the line of the operation. Distal to this lie cartilage cells in clumps surrounded in places by calcified matrix. Proximally well-marked cartilage columns are visible proceeding in a regular fashion to the metaphysis (Pl. 2, fig. 16).

*R. 126. Survival 12 weeks. Shortening 3.5 mm.*

The radiographs show slight outward curvature of the forearm bones. The operated ulna is slightly broader than that of the control. The area of the epiphyseal cartilage and the metaphysis appear normal (Pl. 1, fig. 4).

*R. 123. Survival 18 weeks. Shortening 17 mm.*

The radiographs show considerable outward curvature of radius and ulna. The area of the epiphyseal cartilage on the operated side is broad but normally translucent.

*R. 124. Survival 22 weeks. Shortening 2 mm.*

The radiographs of the operated limb show a normal epiphyseal cartilage and metaphysis. The ulna is broader than the control.

The sections show a normal epiphyseal cartilage becoming quiescent. There is a thin reserve area and long partly calcified columns (Pl. 2, fig. 17).

*R. 125. Survival 22 weeks. Shortening 6 mm.*

The outward curvature of the ulna is accentuated compared with the control and the bone is somewhat broader.

The sections show a normal epiphyseal cartilage which appears to be quiescent (Pl. 2, fig. 18).

*R. 127. Survival 22 weeks. Shortening 6 mm.*

The ulna and radius appear curved upon the radiographs, and on the radial border of the ulna is a considerable deposition of new bone. From the radius arises a spur with which the epiphysis of the ulna appears to articulate. The epiphyseal cartilage itself appears narrower than its control.

The sections reveal an irregular epiphyseal cartilage with long columns and very few cells in the reserve zone. This epiphyseal cartilage extends over to and intervenes between the ulnar epiphysis and the radial spur as well as the ulnar metaphysis.

Table 4. *Increase in length of ulna following excision of proximal fragment of epiphyseal cartilage*

No.	Age at operation (days)	Post-operative survival (weeks)	Growth in first 3 weeks		Total post-operative growth	
			Operated (mm.)	Control (mm.)	Operated (mm.)	Control (mm.)
123	21	18	10	10.5	20	37
124	21	22	11.5	12.5	43	45
125	21	22	10	12	37	43
126	21	12	11	12.5	32.5	36
127	21	22	12.5	12.5	38	44
230	16	5	*	*	10	15
232	16	4	*	*	9	12

\* Not measured.

## DISCUSSION

Following complete excision of its distal epiphyseal cartilage, the ulna at first increases in length at a rate only slightly slower than its control (Table 1). The mutual attachment of the radial and ulnar epiphyses permits the growing radius to distract the fragments of the ulna, and this distraction is facilitated by the connective tissue which occupies the operation site at this period. After the first 3 weeks further growth is unusual, and analysis of the contributions from each end of the bone often shows that the retardation of growth at the distal end is associated with a stimulation at the proximal end (Table 2). In most animals a bony bridge develops between the epiphysis and shaft of the ulna. Within and around this area islands of cartilage may be seen, and these islands may be organized into mature structures resembling the normal epiphyseal plate, although often completely surrounded by bone and thus unable to contribute to further lengthening of the ulna. These islands are sometimes associated with a spur arising from the radius at a point where the periosteum may be damaged during the operation. In two animals the cartilage developed at this point is organized into a broad plate of epiphyseal cartilage which was associated with normal growth in length until the biopsy.

Excision of the distal segment of cartilage containing the reserve zone produces results essentially similar to those of the complete excision. Considerable shortening of the ulna commonly follows (Table 3). Reformation of the epiphyseal cartilage is sometimes associated with a prominent spur from the radius, against which growth in length may occur. Normal growth shown by one animal (R. 114) may be associated with incomplete removal of the reserve area. In the absence of the reserve zone, the cartilage columns appear to become isolated and fragmented rather than replaced by bone from the metaphysis. They are seen to persist for periods of up to 14 days after the operation. In those animals in which the area containing the cartilage columns had alone been removed, shortening is, on the whole, slight. Examination of the sections in which reformation of the epiphyseal cartilage had occurred suggests that the new cartilage columns are often derived from the metaphysis, rather than from an extension proximally of the reserve zone of cartilage.

In a preliminary contribution (Ring, 1953) it was concluded that regeneration following complete excision of the epiphyseal cartilage did not occur. The further evidence now presented suggests that under favourable circumstances a new epi-

physeal cartilage may differentiate from the cartilage in the connective tissue which unites the two bony fragments. This cartilage may function in the normal growth of the bone provided that it appears before bony union has occurred at any point between epiphysis and diaphysis. Under these circumstances it is clear that the ulna provides a favourable site for regeneration since the fragments remain separated for some 3 weeks after operation. Banks & Compere (1941) were unable to find any evidence of regeneration following a similar excision of the epiphyseal cartilage of the femur, where bony union between the two fragments was rapid. Selye (1934), and later Lacroix (1951), removed the distal end of the femur of the rat by an incision passing above the epiphyseal line. Both authors found the end of the femur covered by a mass of cartilage indistinguishable from epiphyseal cartilage. This mass of cartilage also, however, resembles the articular cartilage of young bone. The proof of its nature lies finally in studies of the further growth of the bone, and although this is said to proceed normally for some time, no measurements are recorded in these communications.

The experiments of Hellstadius (1947) fall into two groups. In the first group resection of the distal epiphysis and epiphyseal cartilage was performed. In one animal this was followed by regeneration of the epiphyseal cartilage and a considerable increase in length, in the other two, slight new bone formation occurred within the connective tissue distal to the cut diaphysis. The second group of animals, five in number, was subjected to a subperiosteal resection of the distal end of the ulna including a variable length of the shaft, the epiphyseal cartilage, and the epiphysis. Each of these animals showed a considerable post-operative elongation of the ulna. Since, however, the attachment of the distal end of the ulnar periosteum to the radial epiphysis was presumably undisturbed, this periosteum was stretched by the lengthening radius. The considerable new bone formation recorded in three of these animals appears to have been periosteal in origin. Hellstadius concluded that longitudinal growth could occur in the absence of the epiphyseal cartilage. The results recorded, however, are largely dictated by the growth of the radius, and represent not active growth, but ossification within a stretching scar, or deep to a stretching periosteum. The role of the epiphyseal cartilage is not merely to prevent bony union between metaphysis and epiphysis, but is itself responsible for active bone growth. Indeed, the close relationship between histological reformation of an epiphyseal cartilage and the growth of bone demonstrated radiologically confirms this view.

Experiments involving excision of one part of the epiphyseal cartilage throw some light on the localization of function within this area. Little disturbance of growth is produced by removal of the cartilage columns, the degenerating cartilage cells, and the edge of the metaphysis. Renewal of this area, however, often appears from the metaphyseal end, and examination of the whole series of sections produces no fresh evidence to support the current theories that growth in length is associated with a division of cartilage cells in the reserve area followed by their arrangement in columns, calcification of the interstitial substance and replacement by bone. It is clear indeed, that growth in length associated with the spread of calcification within the scar tissue may often occur, although the growth is primarily active in the radius, and only passive in the scar tissue of the ulna. Observations by Sissons (1953)



appear to suggest that complete replacement of the cell columns in the epiphysis of the rabbit should occur within 24-48 hr. Persistence of these columns for as long as 14 days after the reserve zone has been removed implies that their invasion from the metaphysis depends upon the integrity of the reserve zone, the activities and presence of which determine the rate and mode of bone growth. It is uncommon, however, to see mitotic figures in this reserve area, although cellular division must indeed be rapid and frequent. It has been suggested that these cells divide amitotically, although Lacroix states that numerous mitotic figures may be observed in sections cut transversely through the reserve zone.

One other feature of bone growth emerges from this work. Where the ulna becomes shorter than its control, it is almost always broader, and this increase in diameter is often associated with clubbing in the metaphyseal region. Occasionally this clubbing is associated with a proximal extension of the epiphyseal cartilage, when this is present, around the edges of the bone. More commonly, however, this new bone is of periosteal origin. Increase in bone diameter in this way is explicable on mechanical grounds alone. Where the bone is growing less rapidly in length, the periosteum, attached to its ends, is subject to less distraction, and, it may be assumed, has a greater concentration of osteoblasts for each unit of length, and thus a greater potentiality for transverse growth.

#### SUMMARY

1. The effect upon bone growth of partial or complete excision of the ulnar epiphyseal cartilage has been studied.

2. Complete excision of the epiphyseal cartilage is followed by regeneration which is, on the whole, abortive. Early post-operative increase in length is due to distraction by the radius: later increases can be correlated with the extent of cartilage regeneration.

3. Bony union between the diaphysis and epiphysis or bony encapsulation of the new cartilage prevents further growth.

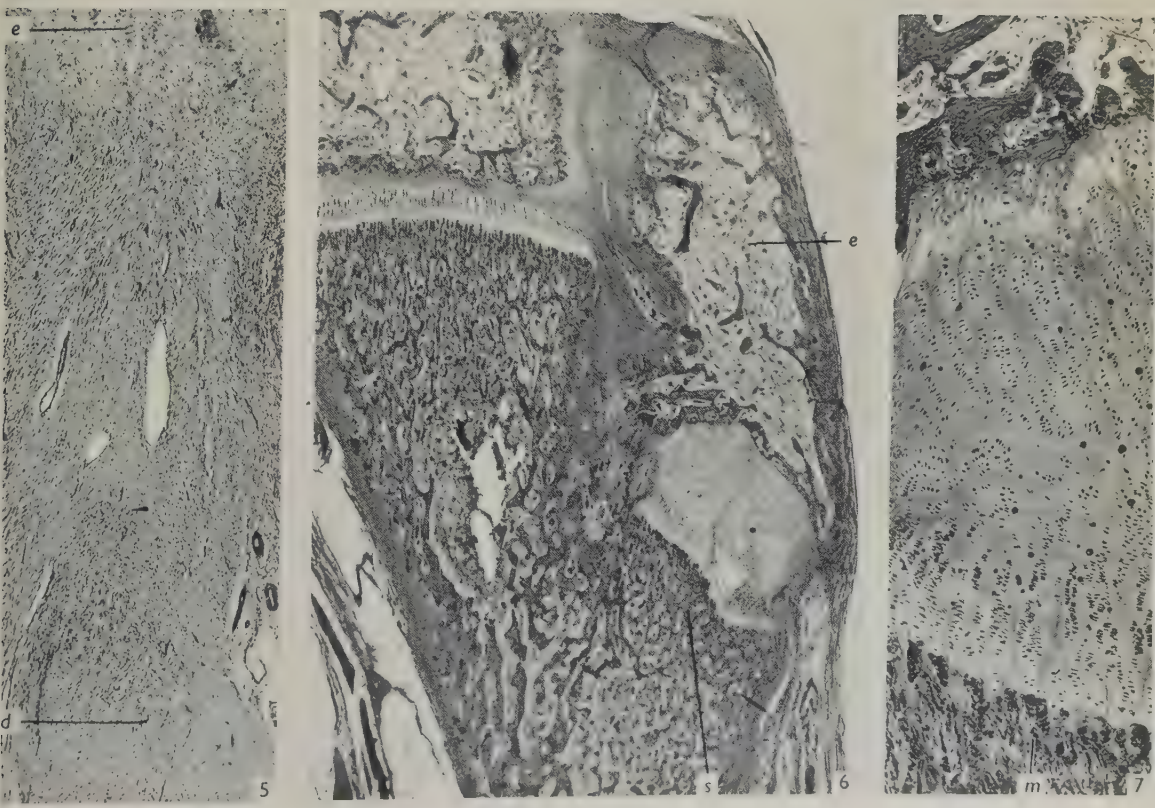
4. Excision of the reserve zone arrests growth. Preservation of the cartilage columns for periods of up to 14 days throws some doubt upon the accepted mode and speed of their replacement.

5. Excision of cartilage columns alone produces no significant disturbance of growth. The columns appear to reform from the metaphyseal side.

I wish to acknowledge with gratitude the encouragement and constructive criticism of Prof. W. J. Hamilton. I am indebted to Mr R. J. McCulloch for his technical assistance and to Mr E. V. F. Pittock for the photography.

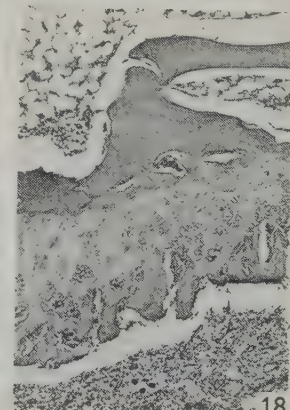
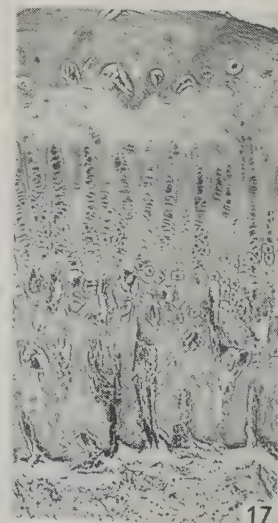
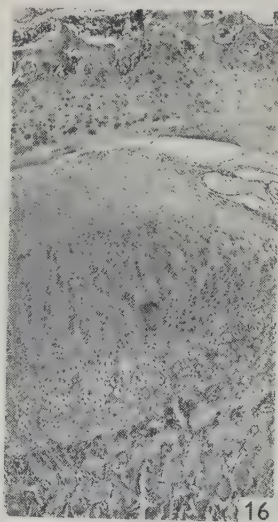
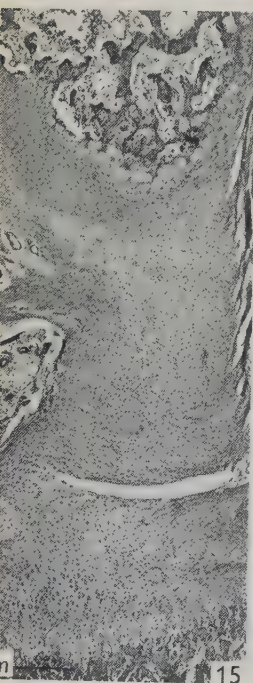
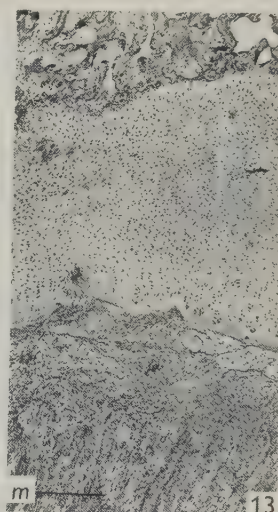
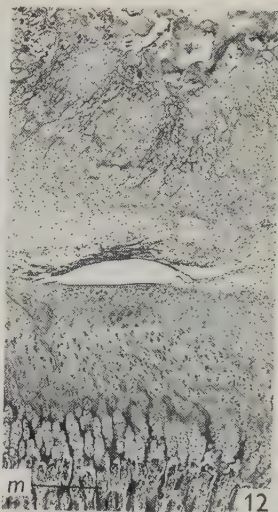
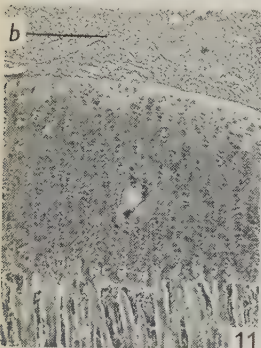
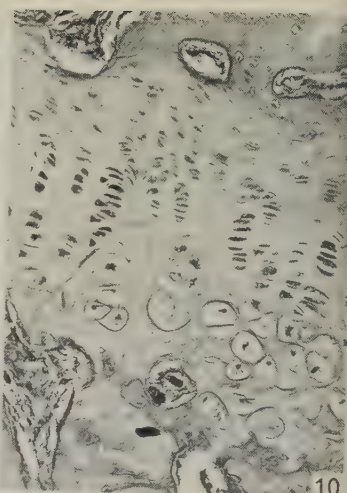
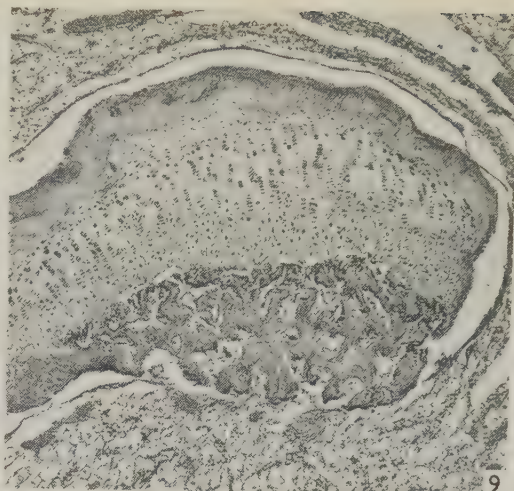
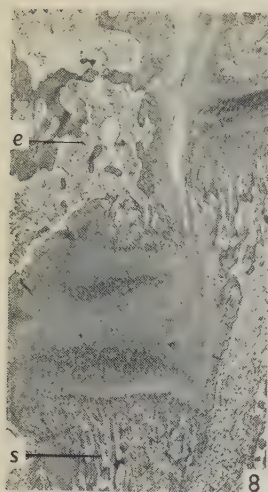
#### REFERENCES

- BANKS, S. W. & COMPERE, E. L. (1941). Regeneration of epiphyseal cartilage. *Ann. Surg.* **114**, 1076-1084.
- HELLSTADIUS, A. (1947). An investigation, by experiments on animals, of the role played by the epiphyseal cartilage in longitudinal growth. *Acta chir. scand.* **95**, 156-166.
- LACROIX, P. (1951). *The Organisation of Bones*. London: J. and A. Churchill Ltd.
- RING, P. A. (1953). Epiphyseal excision and transplantation in the rabbit. *Proc. Anat. Soc. J. Anat., Lond.*, **87**, 459.



RING—EFFECTS OF EXCISION OF EPIPHYSEAL CARTILAGE OF THE RABBIT







- SELYE, H. (1934). On the mechanism controlling the growth in length of the long bones. *J. Anat., Lond.*, **68**, 289-292.
- SISSONS, H. A. (1953). Experimental determination of rate of longitudinal bone growth. *J. Anat., Lond.*, **87**, 228-236.

## EXPLANATION OF PLATES

### PLATE 1

- Fig. 1. R. 164. Five weeks after complete excision of epiphyseal cartilage. A spur from the radius separates the two fragments of the ulna. Growth is proceeding between this spur and the epiphysis of the ulna.
- Fig. 2. R. 161. Six weeks after complete excision of epiphyseal cartilage. Marked curvature of the radius with clubbing of its metaphysis is associated with considerable shortening (8.5 mm.) of the ulna. There is a thin epiphyseal line common to both radius and ulna.
- Fig. 3. R. 113. Thirty weeks after excision of distal segment of epiphyseal cartilage. Marked curvature of radius and ulna with clubbing of both bones. No epiphyseal line is visible.
- Fig. 4. R. 126. Twelve weeks after excision of proximal segment of epiphyseal cartilage. Normal growth has occurred.
- Fig. 5. R. 115. Nine days after complete excision of epiphyseal cartilage. The gap between epiphysis (*e*) and diaphysis (*d*) is occupied by a vascular fibrous tissue.  $\times 35$ .
- Fig. 6. R. 164. Five weeks after complete excision of epiphyseal cartilage. The radial epiphysis is normal. A spur (*S*) on the radius is capped by cartilage which articulates with the ulnar epiphysis (*e*).  $\times 15$ .
- Fig. 7. R. 164. The cartilage and spur of the radius under higher power. The arrangement of the cartilage columns and the modification of the radial shaft into a metaphysis (*m*) can be seen.  $\times 60$ .

### PLATE 2

- Fig. 8. R. 165. Five weeks after complete excision of epiphyseal cartilage. There is a broad band of cartilage uniting the epiphysis (*e*) of the ulna to a spur (*s*) on the radius. Well-marked cartilage columns are visible at both ends of this area.  $\times 12$ .
- Fig. 9. R. 162. Nine weeks after complete excision of epiphyseal cartilage. A small, well-organized growth cartilage is set on a bony shelf projecting into the medullary cavity of the ulna.  $\times 45$ .
- Fig. 10. R. 83. Ten weeks after complete excision of epiphyseal cartilage. A thin cartilaginous area lies between epiphysis and shaft.  $\times 182$ .
- Fig. 11. R. 228. One week after excision of distal segment of epiphyseal cartilage. The operation area is occupied by blood clot (*b*). Below this the cartilage columns appear unchanged.  $\times 42$ .
- Fig. 12. R. 207. Two weeks after excision of distal segment of epiphyseal cartilage. The space in the centre represents the proximal limit of the excised area. On the metaphyseal side (*m*) lie well-organized cartilage columns, on the epiphyseal side are clumps of cartilage cells.  $\times 27$ .
- Fig. 13. R. 201. Six days after excision of proximal segment of epiphyseal cartilage. The excised area is replaced by fibrous tissue into which extend columns of bone from the metaphysis (*m*).  $\times 30$ .
- Fig. 14. R. 225. Two weeks after excision of proximal segment of epiphyseal cartilage. Long columns of cartilage cells occupy the operation site. In this section they do not reach the metaphysis.  $\times 30$ .
- Fig. 15. R. 232. Four weeks after excision of proximal segment of epiphyseal cartilage. The distal limit of the excision is indicated by the gap, proximal to which lies a number of cartilage cells, in part arranged in columns, and apparently coming from the metaphyseal region (*m*).  $\times 30$ .
- Fig. 16. R. 230. Five weeks after excision of proximal fragment of epiphyseal cartilage. The distal limit of the excision is represented by the gap passing across the section. Proximal to this is a well-organized growth cartilage.  $\times 33$ .
- Fig. 17. R. 124. Twenty-two weeks after excision of proximal segment of epiphyseal cartilage. The structure of the epiphyseal cartilage appears normal although the cartilage columns are rather long.  $\times 75$ .
- Fig. 18. R. 125. Twenty-two weeks after excision of proximal segment of epiphyseal cartilage. The growth cartilage shows a normal, quiescent structure.  $\times 42$ .

## SOME OBSERVATIONS ON THE INTRACELLULAR LIPID IN THE KIDNEY OF THE CAT

BY MARY C. LOBBAN

*Physiological Laboratory, University of Cambridge*

Smith (1920) gave the first detailed account of the occurrence of peculiar intracellular lipid deposits in the kidney of the normal adult cat. Smith noted that these fatty deposits were confined to the cells of the kidney cortex, and also that the amount of fat present did not vary with the depot fat, but did vary with age, being least in the young kitten and greatest in the old cat. At a later date, Modell (1933) confirmed and enlarged upon Smith's findings. He demonstrated that the intracellular lipid deposits were confined to 'the convoluted portions of the nephron', and also that it was not the kidney of the very old cat, but that of the pregnant female, which showed the presence of the greatest amount of lipid. No other variation in the amount of lipid present which could be correlated with sexual activity was mentioned by either Smith or Modell, and no further indication was given of any sex differentiation. Also, as all the sections on which these early observations were made were treated with general fat-colorants only, such as Scarlet Red and Sudan III, no specific information can now be gathered as to the type of fat present.

More recently, evidence has been accumulating for the existence of a marked difference between the kidneys of the two sexes in species other than the cat, not only in so far as the actual size and weight of the organ is concerned (Selye, 1939, 1940; Martens & Nylen, 1946) but also with regard to intracellular reactions. Such work includes that of Eschenbrenner & Miller (1945) on sex differences to chloroform-induced kidney necrosis in mice; Kochakian (1944, 1945) on the effects of castration and hormone administration and the distribution of phosphatases in the mouse kidney; and Oster & Oster (1946) on changes in the cell aldehydes of the rat kidney during the oestrous cycle. Modell (1933) had also suggested that it might be worth while to investigate the kidneys of carnivores other than the cat for the presence of intracellular lipids, and in this connexion MacNider (1945) has recently reported on their occurrence in the dog, while Hewer, Matthews & Malkin (1948) have demonstrated the presence of lipids in the kidney cells of the tiger, lion and other large wild cats. Smith, in her original paper on this subject, indicated that varying quantities of lipids could be demonstrated in the kidneys of the dog, rat and rabbit, although the general picture presented by the kidneys of these species was in no case as striking as that found in the kidney of the cat. Gairns & Morrison (1949) have recently reinvestigated the lipid deposits in the cat's kidney, with results agreeing, in the main, with those already described by the earlier workers.

It would seem that two main points emerge from the work described in the preceding paragraphs. First, that the occurrence of intracellular lipids, in varying amounts, is a perfectly normal finding in the kidney cortex of the adult cat; and, secondly, that in rodents cellular differences may be demonstrated between the kidneys of the two sexes, and in the kidney of the female at different stages of the

oestrous cycle. The present work was undertaken with the object of seeing whether any connexion could be established between these two main observations, and whether the amount of lipid in the cells of the cat's kidney does, in fact, bear any relationship to the sexual state of the animal. Since the kidney bears a close phylogenetic and embryological relationship to the adrenal cortex and to the gonads, both of which produce appreciable amounts of lipids in the form of steroids, it was also thought that part, at least, of the kidney lipids might well be steroid in character. In an attempt to produce evidence in support of this hypothesis, the work was extended to include a histochemical investigation of the nature of the intracellular kidney lipids.

## METHODS

*Experimental animals.* The kidneys of fifty-five adult cats and thirty-two kittens of various ages were examined. The adult cats were killed either by bleeding, under suitable anaesthesia, or by the intra-cardial injection of pentobarbitone sodium. All the kittens used in the investigation were killed with intra-cardial pentobarbitone sodium.

*Fixation and staining.* Slices of kidney tissue from both kidneys of each animal were fixed for 3 days in Baker's formal-calcium fixative (Baker, 1944). In some cases, paraffin sections were cut at a thickness of  $7\mu$ , and were stained by the Azan and iron-haematoxylin methods. Frozen sections of all the kidneys were cut at approximately  $15\mu$ , and were treated with Sudan III and haematoxylin and with Sudan Black. The latter is to be preferred as a fat-colorant, as it is believed to be more soluble in lipids other than triglycerides than is Sudan III (Baker, 1946).

*Histochemical methods.* Frozen sections of each kidney were subjected to the Schultze reaction for steroids (adapted from Lison, 1936); the phenyl hydrazine reaction for ketosteroids (Bennett, 1940); and the acid haematin and pyridine extraction tests for the presence of phospholipines (Baker, 1946).

*Determination of sexual activity.* In all the adult cats, the sexual state of the animals was ascertained by direct histological examination of the gonads.

## RESULTS

### A. Presence and distribution of lipids

In all the adult cats, marked individual differences were noted in the occurrence and distribution of the intracellular kidney lipids, although the two kidneys from any one cat always presented the same appearance. Sudanophilic material was present in the kidneys of all normal, sexually active male cats, but only in moderate amounts (Pl. 1, fig. 1). In sexually inactive male cats, however, the kidney cortex showed very heavy lipid deposits. In all the male cats which were examined, the kidney showing the greatest amount of intracellular Sudanophilic material was that of the senescent animal, with testes devoid of any signs of spermatogenesis, a female type of fat distribution over the abdominal muscles, and abnormally-developed mammae (Pl. 1, fig. 2). An appreciable increase above normal in the amount of intracellular kidney lipid was also found in the young adult male cat, three weeks after surgical castration (Pl. 1, fig. 3).

The kidneys of the female cats showed a much wider variation in the amount of



intracellular lipid present than did those of the males. No lipid, or only a trace, was found in the kidney of the anoestrous female (Pl. 1, fig. 4), while moderate amounts were demonstrable in the kidneys of pro-oestrous and oestrous animals (Pl. 1, fig. 5). The kidneys of non-pregnant cats in the luteal phase of the oestrous cycle showed fairly heavy lipid deposits (Pl. 1, fig. 6) while, as stated by Modell in the early work on the subject, the most heavily lipid-laden kidney of all was that of the pregnant cat in the early stages of pregnancy (Pl. 1, fig. 7).

In all the adult cats where intracellular lipids were present, the lipids were typically confined to the convoluted portions of the nephron, with the exception of the junction of the proximal convoluted tubule with the descending limb of Henle's loop. The greater part of the lipid was present in the cells of the proximal convoluted tubules, the distal convoluted tubules being nearly always completely lipid-free, even in a heavily fat-laden kidney (Pl. 1, fig. 8). Very little or no lipid was found in the cells of the kidney cortex of the young kitten (Pl. 2, fig. 9) and the beginning of a typical adult type of fat distribution was rarely found in kittens of less than 2 months of age, and often not until much later, e.g. 4-5 months. These small amounts of intracellular lipids were only found in the kidneys of male kittens, as a general rule, although in one case distinct traces of lipid were found in the kidneys of two, day-old female kittens from the same litter. Lipids were first seen in the form of discrete droplets near the junction of the proximal convoluted tubule with the descending limb of Henle's loop, and it would appear that the lipids extend back towards the glomerulus with increasing age. In older kittens, where traces of lipid were present, the lipid droplets were more readily coloured with Sudan Black than with Sudan III.

A careful examination of similar sections gave an indication of the presence of two types of Sudanophilic material within the cells of the kidney tubules. One type occurred in the form of relatively large droplets ( $2-5\mu$  in diameter) and was coloured both by Sudan III and Sudan Black, while the other, which occurred as much finer droplets, was coloured by Sudan Black only. The two types varied in parallel fashion, except in the kidney of the young male kitten which was just beginning to accumulate lipids, where the type staining with Sudan Black only was often present without the Sudan III staining type. It was also observed that, in the moderately lipid-laden kidney, the lipid droplets were situated for the most part towards the base of the cells, away from the brush border and the lumen of the tubule. Occasionally, large Sudanophilic globules were seen lying free in the lumen, but these were invariably associated with the presence of damaged cells, and it is thought that they do not occur naturally in this situation, but had most probably been extruded during the processes of freezing and sectioning. Modell (1933) stated that varying quantities of lipids could be demonstrated in the urine of the cat, but all attempts to confirm this observation during the present work have been entirely unsuccessful, even when the urine was obtained from cats which were subsequently shown to possess heavily fat-laden kidneys. If this finding is correct, it would seem that the domestic cat differs markedly from the large wild cats in this respect, as the urine of the tiger has been shown to contain large quantities of lipid material (Hewer *et al.* 1948).

*B. Histochemical observations*

All three histochemical tests were carried out on formal-calcium fixed material. The frozen sections used for the Schultze and phenyl-hydrazine reactions were thoroughly washed in several changes of distilled water, before being treated with the appropriate reagents. The phenyl-hydrazine and acid haematin and pyridine extraction tests were carried out exactly as described by Bennett (1940) and Baker (1946) respectively, but certain modifications were made in the technique of the Schultze reaction. In this test, it was found that a reagent consisting of 67 % concentrated sulphuric acid and 33 % glacial acetic acid (v/v) was more effective than the original Schultze reagent (Lison, 1936), which consisted of equal parts of the two acids, and also that the rapidity with which the colours developed and the final intensity of the colours were further improved if the reagent was kept for at least 2 days in a loosely-stoppered bottle, before use. All the sections used for the Schultze reaction were first incubated for 24 hr. in a 2.5 % solution of iron alum at 37° C.

In all the kidney sections, the acid haematin and pyridine extraction techniques yielded uniformly negative results, while in the case of young kittens and anoestrous female cats, where the kidney sections contained no Sudanophilic material, the Schultze and phenyl-hydrazine reactions were also negative. In all the kidneys where Sudanophilic material was present, however, the phenyl-hydrazine reaction was definitely positive, and these sections also yielded a colour reaction with the Schultze reagent. In all cases, the positive reactions obtained with these reagents were confined to the kidney cortex, and the extent and intensity of the colours obtained varied according to the amount of Sudanophilic material which had previously been shown to be present. The colour which was produced in the Schultze reaction was not the intense blue-green which is given with this reagent by cholesterol and its esters, the androgens, and certain of the adrenal cortical steroids, but was a definite pink, which developed relatively slowly, and remained stable for periods extending up to 3 hr. This pink colour could be exactly reproduced in the test-tube by adding concentrated sulphuric acid to an equal volume of a chloroform solution of oestrone or oestradiol (1 mg./ml.) to which a trace of cholesterol had been added, and diluting the resulting brilliant yellow, fluorescent solution with three parts by volume of distilled water. The colour so obtained was intensified by the addition of a few drops of a 2.5 % solution of iron alum. It is thought that this pink coloration is closely allied to the red colour given by the naturally occurring oestrogens with the Kober colour reaction (Kober, 1931; Brown, 1952). The fact that a pink colour identical with that observed in the lipid-containing kidney has also been obtained in the follicular fluid and zona granulosa of the Graafian follicles in the Schultze-treated ovary, and in the Sertoli cells of the testis, provides additional support for this view.

The histochemical results, although by no means conclusive, do seem to give some indication that part of the intracellular lipids in the kidney cortex of the cat are possibly steroid in character. If this be so, the steroids present are not likely to be cholesterol alone, the androgens, or the main adrenal cortical steroids, all of which give an intense green or blue-green colour both in Schultze-treated sections and in the test-tube with concentrated sulphuric acid, water and iron alum. There is, how-

ever, a distinct possibility that they may be allied to the naturally occurring oestrogens. Further histochemical investigations are now being undertaken in an attempt to clarify this point.

#### DISCUSSION

The observations described in this work show that intracellular lipid, in varying amounts, is present in the kidneys of all adult cats, except in the anoestrous female. Moderate amounts of lipids are present in the kidney cortex of the oestrous female and the normal, sexually active male, while a somewhat heavier deposition of lipids is found in the kidneys of the pseudopregnant female and the surgically castrated male. The heaviest lipid deposits, however, are found in the kidney of the pregnant female and the senescent, sexually inactive male. In every case, the lipids are confined to the cells of the kidney cortex, and there is some indication that they may be, at least in part, of a steroid nature.

In the female cat, it would appear that the amount of intracellular lipids present in the kidney cortex at any one time bears a direct relationship to the sexual state of the animal. The anoestrous female has no lipid (or, occasionally, only a trace) in the kidney cortex, whereas the oestrous female shows an appreciable amount, the lipid droplets being scattered throughout the epithelium of the proximal convoluted tubules. The amount of lipid present increases throughout oestrus, and reaches a maximum in the luteal phase of the cycle; if the cat becomes pregnant, the lipid deposits are very heavy indeed, the kidney cortex appearing to be packed with Sudanophilic material. It is presumed that towards the end of pregnancy or pseudopregnancy the amount of lipid present in the kidney tubule cells begins to diminish, and eventually the cat returns to the anoestrous state, with a lipid-free kidney. The diminution in kidney lipid at this stage has not yet been observed, but adult, anoestrous cats with the scars of old corpora lutea in their ovaries (and, in one case, a cat which was known to have given birth to a litter of four kittens some 15 weeks previously) have been found to possess kidneys which are just as free of lipids as are those of the young female which has not yet reached sexual maturity. It would seem, then, that the laying down of lipids in the kidney of the female cat occurs only at times when there are greatly increased amounts of the female sex hormones in the circulation. If it is believed that these female hormones are the cause of lipid accumulation in the kidney, then progesterone has a more marked effect in this respect than have the oestrogens, since the deposition is considerably greater in the luteal phase of the cycle than in the follicular phase.

The picture presented by the lipids in the kidneys of male cats is somewhat more difficult to interpret. The first apparent anomaly arises in the normal, sexually active male, where moderate amounts of intracellular kidney lipids are invariably found. It is well known that small amounts of the female sex hormones are always found in the circulation of the male, and at first sight it might appear permissible to suggest that the kidney lipid content in the male is maintained under the influence of such hormones. When, however, the male kidney is compared with that of the anoestrous female (which animal, even though sexually quiescent, is surely to be regarded as more female, both in general characteristics and in steroid balance, than is the normal male) which is conspicuously free from intracellular lipids, it does



not seem that such an explanation can hold good. It is possible, however, that the hormones responsible may be the anterior pituitary gonadotrophins. It is now generally accepted that the luteinizing hormone (L.H.) of the female is identical with the interstitial cell stimulating hormone (I.C.S.H.) of the male, and it can readily be seen that there may be periods when there is considerably more of this principle in the blood of the normal male than in that of the female, i.e. when the female is in the anoestrous state. Such an explanation could be satisfactorily applied both to the maintenance of the intracellular kidney lipids in the male, and to the laying down of lipids which occurs during sexual activity in the female. The pituitary gonadotrophins could also be the stimulus which causes the laying down of abnormally large amounts of lipid in the kidney of the surgically castrated male; this explanation would be well in accord with the increase in the number of  $\beta$  cells which is regularly observed in the anterior pituitary after castration.

The increase in the amount of kidney lipids observed in the senescent male cat cannot, however, be ascribed to stimulation by the pituitary gonadotrophins. Indeed, senescence is to be regarded as being directly due to a failure in pituitary gonadotrophin production, histological examination of the testes of senescent animals showing complete absence of spermatogenesis together with the presence of shrunken, inactive interstitial cells, the latter appearing to be conspicuously poor in steroid content. The Sertoli cells in such a testis, however, are remarkable for their very large size and greatly increased lipid content. The lipid droplets in these cells have been found to yield a pink colour with the Schultze reagent which is identical with that already described in the cells of the lipid-containing kidney, and it is suggested that, under these conditions, the Sertoli cells may well be the source of appreciable quantities of oestrogens. This view is supported by the work of Huggins & Moulder (1945) who have correlated the occurrence of Sertoli cell tumours with feminism in the male dog; the picture presented by such dogs, with their tendency to lay down increased amounts of subcutaneous fat, loss of sexual function, attractiveness to other males, and abnormally developed mammae, greatly resembles the condition observed in the senescent male cat. No data is available on the condition of the dog's kidney under these circumstances. In the senescent cat, however, it is suggested that the very heavy lipid deposits which are observed in the cells of the kidney cortex may be due to increased amounts of female steroids in the circulation which, in turn, may be produced by the hyperactive Sertoli cells. There is, however, the possibility that if the kidney steroids are indeed of the oestrogen type, the reverse argument may be true, in that it is the kidney oestrogens which are producing the changes in the testis, secondary sexual characteristics and in the adrenal cortex (Lobban, 1952). If this be so, no explanation can, as yet, be offered for the increased laying down of lipids in the kidney of the senescent male cat. It is clear that further work must be carried out on the hormone balance in the male cat before this problem can be solved: such work is now in progress.

## SUMMARY

1. A study has been made of the occurrence and distribution of intracellular lipid in the kidneys of fifty-five adult cats and thirty-two kittens.

2. It has been found that a marked increase in the laying down of intracellular kidney lipids is associated with the loss of sexual function in the male cat and with the luteal phase of the oestrous cycle in the female.

3. Histochemical evidence has been put forward which suggests that part, at least, of the intracellular lipid is of a steroid nature, and that the steroids present are more likely to be allied to the oestrogens than to the male sex hormones.

4. The significance of these findings and the possible role of the anterior pituitary gonadotrophins in connexion with them has been discussed.

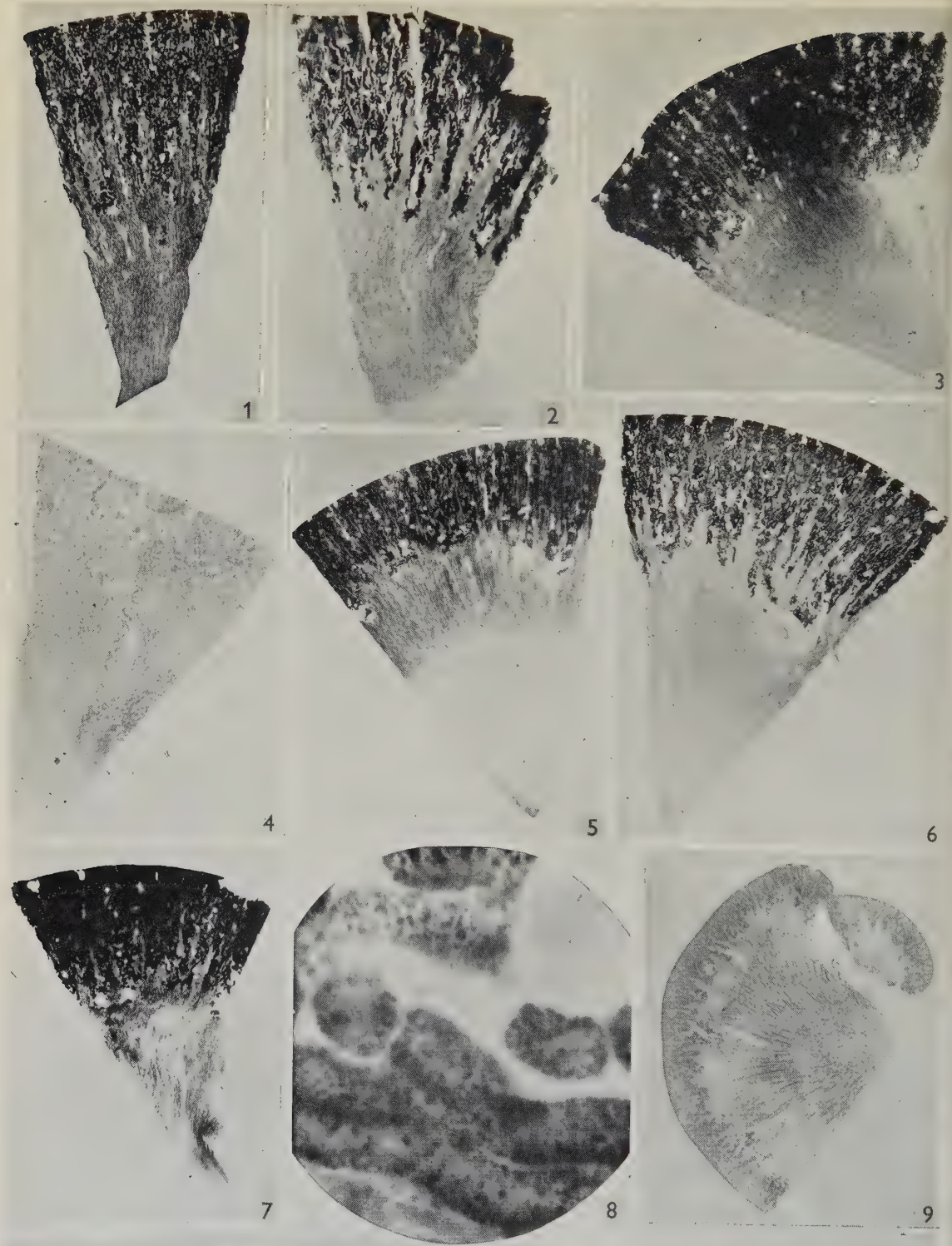
I should like to thank Dr E. N. Willmer for the help and encouragement which he has given me throughout this work, and D. Goode for technical assistance. My thanks are also due to the Agricultural Research Council and to the Royal Society for research grants which made the work possible.

## REFERENCES

- BAKER, J. R. (1944). The structure and chemical composition of the golgi elements. *Quart. J. micr. Sci.* **85**, 1-71.
- BAKER, J. R. (1946). The histochemical recognition of lipines. *Quart. J. micr. Sci.* **87**, 441-470.
- BENNETT, H. S. (1940). The life history and secretion of the cells of the adrenal cortex of the cat. *Amer. J. Anat.* **67**, 151-228.
- BROWN, J. B. (1952). Some observations on the Kober colour and fluorescence reactions of the natural oestrogens. *J. Endocrin.* **8**, 196-210.
- ESCHENBRENNER, A. B. & MILLER, E. (1945). Sex differences in kidney morphology and chloroform necrosis. *Science*, **102**, 302-303.
- GAIRNS, F. W. & MORRISON, S. D. (1949). Lipid in the nephron of the cat. *J. Physiol.* **110**, 17P-18P.
- HEWER, T. F., MATTHEWS, L. H. & MALKIN, T. (1948). Lipuria in tigers. *Proc. zool. Soc. Lond.* **118**, 924-928.
- HUGGINS, C. & MOULDER, P. V. (1945). Estrogen production by Sertoli cell tumors of the testis. *Cancer Res.* **5**, 510-514.
- KOBER, S. (1931). Eine kolorimetrische Bestimmung des Brunsthormons (Menformin). *Biochem. Z.* **239**, 209-212.
- KOCHAKIAN, C. D. (1944). A comparison of the renotropic with the androgenic activity of various steroids. *Amer. J. Physiol.* **142**, 315-325.
- KOCHAKIAN, C. D. (1945). The effect of various steroid hormones on the alkaline and acid phosphatases of the kidney of the mouse. *Amer. J. Physiol.* **145**, 118-122.
- LISON, L. (1936). *Histochimie animale, méthodes et problèmes* (Paris), p. 210.
- LOBBAN, M. C. (1952). Structural variations in the adrenal cortex of the adult cat. *J. Physiol.* **118**, 565-574.
- MACNIDER, W. DE B. (1945). Occurrence of stainable lipid material in renal epithelium of animals falling in different age segments. *Proc. soc. exp. Biol., N.Y.*, **58**, 326-328.
- MARTENS, S. G. R. & NYLEN, B. (1946). On the enlarging effect of desoxycorticosterone acetate on the kidneys of female mice. *Acta anatomica*, **2**, 110-116.
- MODELL, W. (1933). Observations on the lipids in the renal tubule of the cat. *Anat. Rec.* **57**, 13-24.
- OSTER, K. A. & OSTER, J. G. (1946). The specificity of sex hormones on the tissue aldehyde shift in the rat kidney and of fuchsin sulfurous acid reagent on aldehydes. *J. Pharm. & Exp. Therap.* **87**, 306-312.







- SELYE, H. (1939). Morphological changes in female mice receiving large doses of testosterone. *J. Endocrin.* **1**, 208-215.
- SELYE, H. (1940). Production of persistent changes in genital organs of immature female rats treated with testosterone. *Endocrinology*, **27**, 657-660.
- SMITH, C. (1920). A study of the lipid content of the kidney tubule. *Amer. J. Anat.* **27**, 69-94.

EXPLANATION OF PLATE

- Figs. 1-7. Frozen sections of kidneys of adult cats, Sudan Black,  $\times 7$ . Fig. 1. Normal male. Fig. 2. Senescent male. Fig. 3. Male, three weeks after surgical castration. Fig. 4. Anoestrous female. Fig. 5. Oestrous female. Fig. 6. Non-pregnant female in the luteal phase of the oestrous cycle. Fig. 7. Pregnant female, in third week of pregnancy.
- Fig. 8. High-power photomicrograph of kidney of normal male cat, showing lipid droplets in the epithelium of the proximal convoluted tubules. Sudan Black,  $\times 175$ .
- Fig. 9. Frozen section of kidney of 6-week old kitten, Sudan Black,  $\times 7$ .

# ARTERIO-VENOUS ANASTOMOSES IN THE SKIN OF THE HEAD AND EARS OF THE CALF

BY A. MYFANWY GOODALL

*The Hannah Dairy Research Institute, Kirkhill, Ayr*

## INTRODUCTION

Goodall & Yang (1954) have investigated the blood supply in the skin of Ayrshire calves and embryos, but although the method used gave an excellent picture of the general blood supply it was difficult to differentiate between certain vessels, e.g. capillaries and arterio-venous anastomoses.

This paper describes arterio-venous anastomoses found in the skin of the head and ears of Ayrshire calves and the methods used to demonstrate them.

## METHODS

The heads of about a dozen Ayrshire bull calves killed by excess chloroform anaesthesia or pentothal sodium were perfused with Ehrlich's acid haematoxylin following the method of Grant (1930). Attempts at perfusion of individual excised ears were not satisfactory and better results were obtained from perfusing the whole heads through one or both of the carotid arteries. One of the jugular veins was ligated and the other cannulated to allow drainage of the perfused fluids. The ears and pieces of skin from the forehead, cheek and neck were removed.

To trace the blood supply to the ear the heads of two calves were perfused with a synthetic rubber latex (Hycar) coloured with gentian violet. The heads were then fixed in 10 % formalin and subsequently dissected to show the blood supply to the ears.

The ears were cut into four pieces and the skin of both the outer and inner surfaces was dissected leaving the perichondrium with the main blood vessels attached to the cartilage. The perichondrium was then dissected from the cartilage. Each piece of perichondrium was differentiated in acid alcohol if necessary and blued in Scott's tap-water substitute. The piece of perichondrium was then pinned on a wooden block and immersed in increasing concentrations of ethanol to dehydrate it. Mounting on the wooden block prevented it from curling. It was cleared in xylol and mounted in DePeX on a  $3\frac{1}{4} \times 3\frac{1}{4}$  in. lantern slide cover glass. As the perichondrium is fairly thick it was found necessary to build up the corners of the slides with pieces of microslides before covering the mounts with  $3\frac{1}{4} \times 3\frac{1}{4}$  in. no. 1 micro cover-glasses, otherwise the cover glass did not remain flat and air-bubbles penetrated the DePeX.

The skin from the ears and the head was further fixed in 10 % formalin and frozen sections in series parallel to the skin surface were cut at 100 and 200  $\mu$ . The sections were differentiated, blued, cleared and mounted in DePeX.

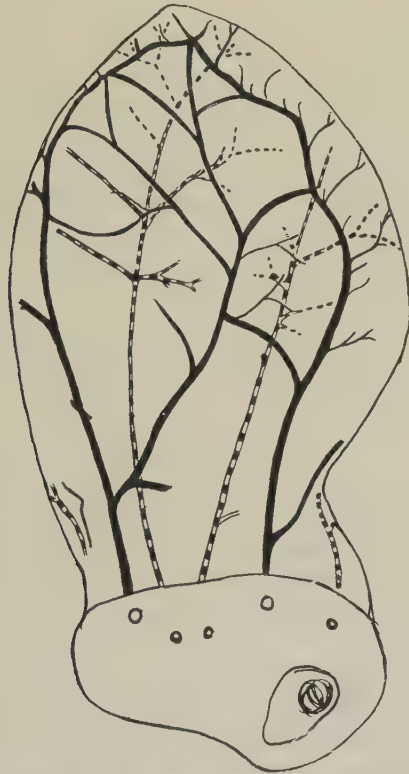
Blocks were cut from several ears obtained from the abattoir. They were fixed in



10% formol saline, embedded in paraffin and serial sections were cut at  $10\mu$ . In some cases alternate slides were stained with haematoxylin and eosin, and orcein and polychrome methylene blue.

#### RESULTS

The blood supply to the helix of the ear is through the posterior auricular artery. From the injected specimens it was observed that in the helix there are four main arteries which lie in the perichondrium of the outer surface of the ear (Text-fig. 1).



Text-fig. 1. A diagram showing the main blood vessels in the perichondrium of the outer surface of the calf's ear. The arteries are dotted and the veins black.

Branches from them supply the skin of the outer surface and also penetrate through the cartilage to anastomose in the perichondrium of the inner surface and then supply the skin of the inner surface. The ear is drained by a system of venous arches culminating in two main veins.

Low power microscopic examination of the perichondrium, whose blood vessels can be regarded as vessels of the first vascular plexus of bovine skin (Goodall & Yang, 1954), showed that there were many arterio-venous anastomoses present. They were sometimes short direct shunts between an artery and vein (Pl. 1, fig. 1) and sometimes were more coiled, dividing into several branches which after a short course joined together to form a common larger vein (Pl. 1, fig. 2), resembling the glomus bodies in the human finger described by Masson (1935) and Popoff (1935).

They were found on both the inner and outer surface of the ear but were more numerous on the outer surface. Maps were made to show the distribution of arterio-venous anastomoses in the perichondrium. It was found that they were more numerous along the margin of the ear than in the centre and more numerous towards the tip than at the base. Pl. 1, fig. 3 shows such a map. Generally only about forty arterio-venous anastomoses were present in any one quarter of the perichondrium. They varied in calibre, their outer diameters ranging from 15 to 90  $\mu$ . They usually occurred in groups along first and second branches of one of the main arteries (Pl. 1, fig. 4).

Examination of the frozen sections of the skin from the ear, forehead and cheek revealed arterio-venous anastomoses in the second vascular plexus of the skin (Goodall & Yang, 1954). They were more numerous than those in the perichondrium but were of finer calibre (Pl. 2, figs. 5-7), and numbered about 10-20/sq.cm.

In the paraffin sections arterio-venous anastomoses in transverse section could be recognized by the thickness of their walls and the presence of epithelioid cells in the media. Pl. 2, fig. 8 shows an arterio-venous anastomosis alongside a small artery. Paraffin sections stained with orcein showed no internal elastic lamina in the arterio-venous anastomoses.

The tongues from several of the perfused heads were removed and frozen sections at 100  $\mu$  were cut in series to determine whether any arterio-venous anastomoses were present, but none were seen.

#### DISCUSSION

Although as a rule capillaries are regarded as being the only communications between arteries and veins the presence of arterio-venous anastomoses in certain organs has for long been known. They were first described by Lealis-Lealis (1707) in the male genital organs. Sucquet (1862) described them in the skin of man in certain body regions, viz. the lips, cheeks, nose and ears and the ends of the fingers and toes. Hyrtl (1862, 1864) described direct communications between the radial artery and the marginal vein in the bat's wing and also in the matrix of the hoof of the horse and of ruminants. Two early workers, Grosser (1902) and Vastarini-Cresi (1902) described the histological structure of the walls of arterio-venous anastomoses. Schumacher (1915) and Clara (1927) showed that such a vessel could be identified by its thick wall and the 'epithelioid' modification of its smooth muscle cells. The muscle cells instead of being spindle-shaped and flat were swollen and possessed intercellular bridges like epithelial cells. The vessels had no internal elastic lamina. The anastomotic vessels which are discussed in this paper appear to have the same structure.

An article by Clark (1938) and a monograph by Clara (1939) give excellent reviews of the literature.

Bennet & Kilham (1940), Nonidez (1942) and Prichard & Daniel (1953) have found arterio-venous anastomoses in several organs, using histological methods. Other workers have perfused organs with glass beads of various diameters and have estimated the size of the largest arterio-venous bridges by measuring the largest beads in the venous outflow (Prinzmetal, Ornitz, Simkin & Bergman, 1948; Tobin & Zariquiey, 1950; Walder, 1952). The more recent work of Gordon, Flasher & Drury

(1953) tends to refute the findings of workers using the glass-bead method although Walder, using that method, confirmed findings made previously by micro-dissection (Barlow, Bentley & Walder, 1951).

The work of Clark & Clark (1930, 1932, 1934*a, b*) and of Grant (1930) has shown that arterio-venous anastomoses are important in the control of blood flow in the rabbit's ear, changing calibre spontaneously and independently of the arteries. By remaining open and so allowing an increase in blood flow through the ear, they maintain local temperatures, preventing frost-bite when environmental temperature is low, and aid in the dissipation of heat when environmental temperature is high.

Grant & Bland (1931) found that during prolonged cooling of the fingers the rise in skin temperature following an initial fall and the rate of this rise are proportional to the number of arterio-venous anastomoses in various skin regions. It may be assumed that the arterio-venous anastomoses as found in the skin of the ears, forehead and cheek of the Ayrshire calf have a similar function. Recent work on the surface temperature of the pinna of the ear of the Ayrshire calf (Findlay & Beakley, 1954) has shown that at an environmental temperature of 10–15° C. the ear temperature undulates around 18° C. If the environmental temperature is raised to 18° C. the temperature of the ear rises quickly to 35° C. and remains at that level. This seems to indicate a great increase in blood flow in the ear at an environmental temperature of 18° C. and it is possible that the arterio-venous anastomoses may play an important part in the mechanism controlling this increase.

Grant (1930) found as many as 100 arterio-venous anastomoses/sq.cm. in the perichondrium of the rabbit's ear. There are not nearly so many in the perichondrium of the calf's ear. This may, however, be explained by the work of Grant & Bland (1931) who found that the number of arterio-venous anastomoses in the skin of the human finger increased from birth to maturity. Thus it is not surprising to find that there are few arterio-venous anastomoses/unit area in the perichondrium of the young calf.

Arterio-venous anastomoses have been found in the tongue of the dog by Brown (1937) and Prichard & Daniel (1953) and also in the tongue of the sheep and the goat by Prichard & Daniel (1953). It has been suggested that they may play some part in the elimination of heat from the animal especially when panting results from excessive heat load. Nevertheless the perfusion method used in the present study has not revealed any anastomoses in the tongue of the Ayrshire calf, which employs polypnoea as a major means of heat dissipation.

#### SUMMARY

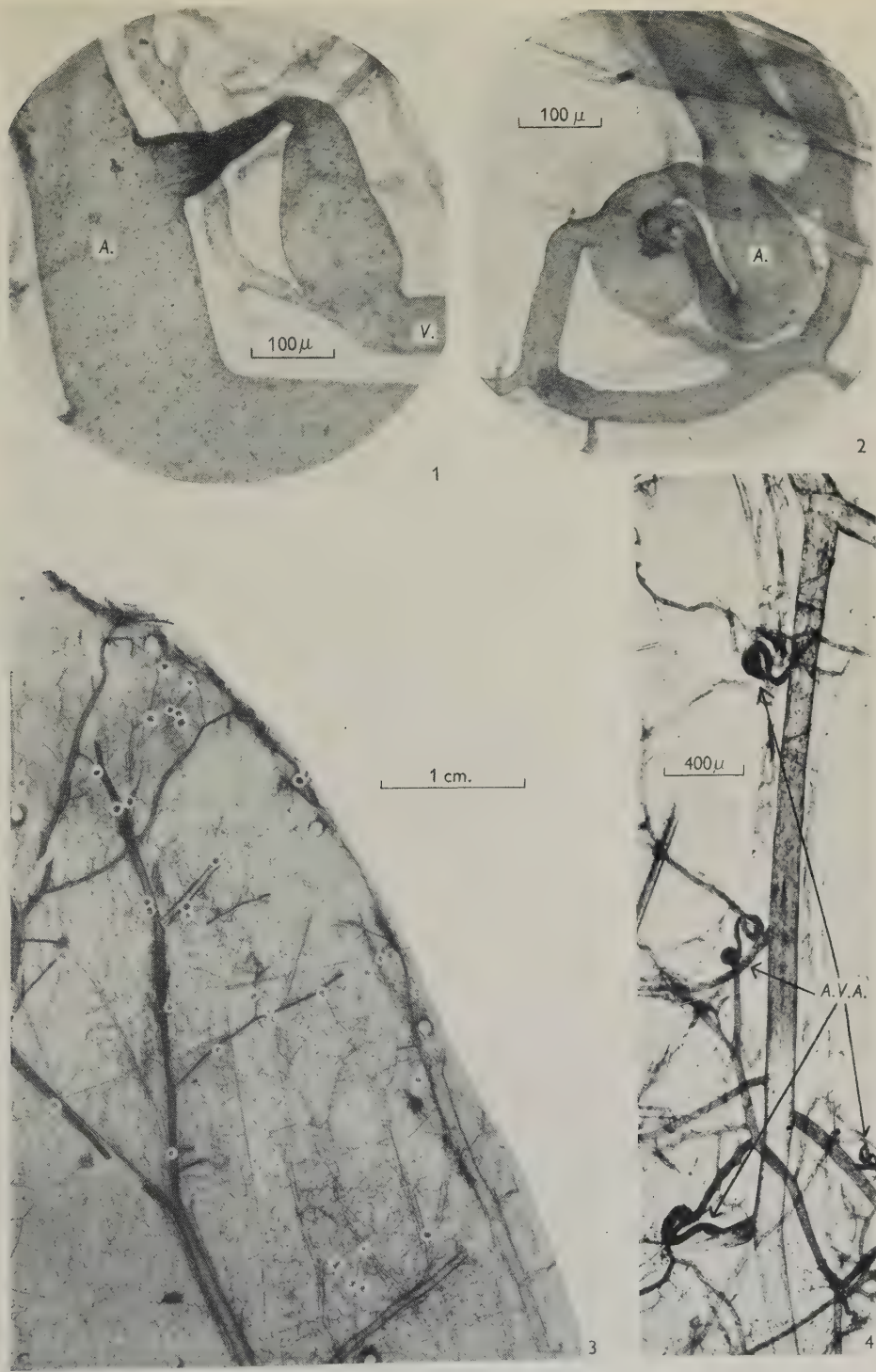
Arterio-venous anastomoses have been found in the skin of the ears, forehead and cheek and in the perichondrium of the ear of the Ayrshire calf. They are direct shunts sometimes straight, sometimes convoluted, joining arteries and veins in both the first and second vascular plexuses of the skin. They have thick walls containing epithelioid cells and have no internal elastic lamina. It is thought that they may play an important role in heat dissipation.



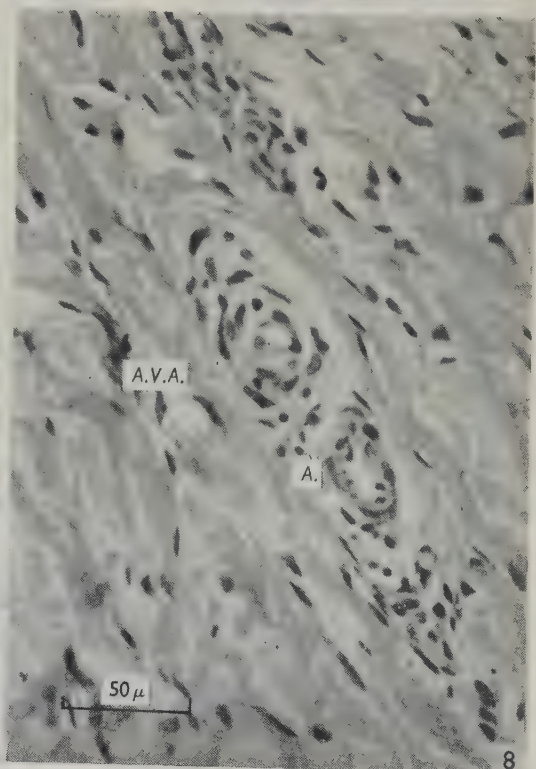
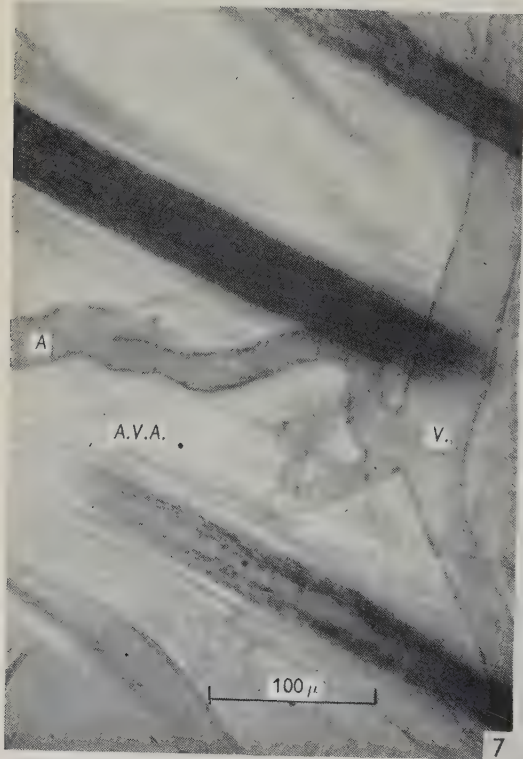
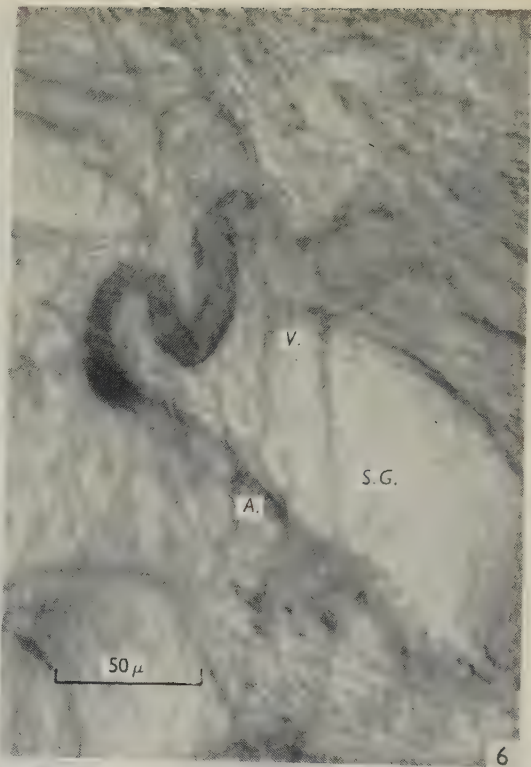
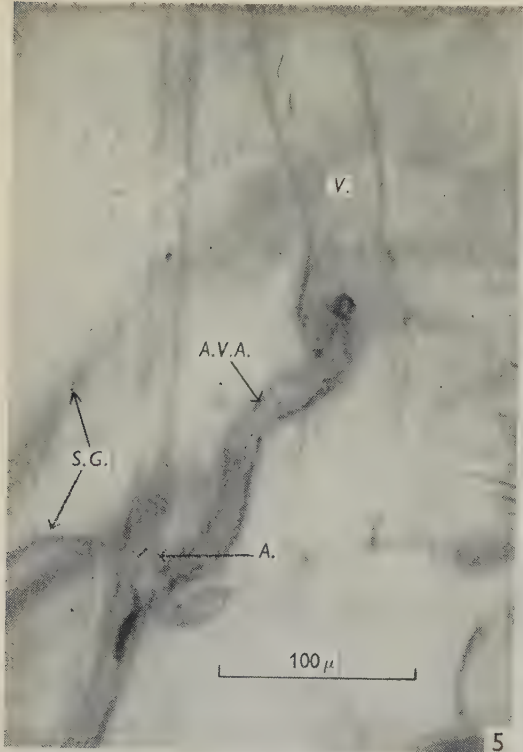
The author wishes to express her thanks to Dr J. D. Findlay and Dr H. S. D. Garven for helpful advice and criticism in the preparation of this paper. She is very grateful to Dr John Bligh for invaluable assistance in carrying out the perfusions and to Misses P. Tait and H. Stewart for technical assistance.

## REFERENCES

- BARLOW, T. E., BENTLEY, F. H. & WALDER, D. N. (1951). Arteries, veins and arterio-venous anastomoses in the human stomach. *Surg. Gynec. Obstet.* **93**, 657-671.
- BENNET, H. S. & KILHAM, L. (1940). The blood vessels of the adrenal gland of the adult cat. *Anat. Rec.* **77**, 447-467.
- BROWN, M. E. (1937). The occurrence of arterio-venous anastomoses in the tongue of the dog. *Anat. Rec.* **69**, 287-292.
- CLARA, M. (1927). Die arterio-venösen Anastomosen der Vögel und Säugetiere. *Ergebn. Anat. EntwGesch.* **27**, 246-301.
- CLARA, M. (1939). *Die arterio-venösen Anastomosen*. Leipzig: J. A. Barth Verlag.
- CLARK, E. R. & CLARK, E. L. (1930). Arterio-venous anastomoses. *Anat. Rec.* **45**, 211.
- CLARK, E. R. & CLARK, E. L. (1932). Observations on living preformed blood vessels as seen in a transparent chamber inserted in the rabbit's ear. *Amer. J. Anat.* **49**, 441-477.
- CLARK, E. R. & CLARK, E. L. (1934a). Observations on living arterio-venous anastomoses as seen in transparent chambers introduced into the rabbit's ear. *Amer. J. Anat.* **54**, 229-286.
- CLARK, E. R. & CLARK, E. L. (1934b). The new formation of arterio-venous anastomoses in the rabbit's ear. *Amer. J. Anat.* **55**, 407-467.
- CLARK, E. R. (1938). Arterio-venous anastomoses. *Physiol. Rev.* **18**, 229-247.
- FINDLAY, J. D. & BEAKLEY, W. R. (1954). The effect of environmental temperature and humidity on the ear temperature of Ayrshire calves. *J. agric. Sci.* (in the Press).
- GOODALL, A. M. & YANG, S. H. (1954). The vascular supply of the skin of Ayrshire calves and embryos. *J. agric. Sci.* **44**, 1-4.
- GORDON, D. B., FLASHER, J. & DRURY, D. R. (1953). Size of the largest arterio-venous vessels in various organs. *Amer. J. Physiol.* **173**, 275-281.
- GRANT, R. T. (1930). Observations on direct communications between arteries and veins in the rabbit's ear. *Heart*, **15**, 281-303.
- GRANT, R. T. & BLAND, E. F. (1931). Observations on the arterio-venous anastomoses in human skin and in the bird's foot with special reference to the reaction to cold. *Heart*, **15**, 385-406.
- GROSSER, O. (1902). Ueber arterio-venöse Anastomosen an den Extremitätenenden beim Menschen und den krallentragenden Säugethieren. *Arch. mikr. Anat.* **60**, 191-206.
- HYRTL, J. (1862). Anatomical notes. *Nat. Hist. Rev., Lond.*, **2**, 99-100.
- HYRTL, J. (1864). Neue Wundernetze und Geflechte bei Vögel und Säugetieren. *Denkschr. Akad. Wiss. Wien.* **22**, 113-153.
- LEALIS-LEALIS (1707). Cited by Vastarini-Cresi.
- MASSON, P. (1935). Les glomus cutanés de l'homme. *Bull. Soc. franc. Derm. Syph., Reunion, Strasbourg*, 1174-1194.
- NONIDEZ, J. F. (1942). Arterio-venous anastomoses in the sympathetic chain ganglia of the dog. *Anat. Rec.* **82**, 593-604.
- POPOFF, N. W. (1935). Recherches sur l'histologie des anastomoses arterio-veineuses des extrémités et sur leur rôle en pathologie vasculaire. *Bull. d'Hist. Appl.* **12**, 156-171.
- PRICHARD, M. M. L. & DANIEL, P. M. (1953). Arterio-venous anastomoses in the tongue of the dog. *J. Anat., Lond.*, **87**, 66-74.
- PRINZMETAL, M., ORNITZ, E. M. JR., SIMKIN, B. & BERGMAN, H. C. (1948). Arterio-venous anastomoses in liver, spleen & lungs. *Amer. J. Anat.* **152**, 48-52.
- SCHUMACHER, S. (1915). Arterio-venöse Anastomosen in den Zehen der Vogel. *Arch. mikr. Anat.* **87**, 309-340.
- SUCQUET, J. P. (1862). *Anatomie et physiologie. Circulation du sang. D'une circulation derivative dans les membres et dans la tête chez l'homme*. Paris: Delahaye.
- TOBIN, C. E. & ZARIQUIEY, M. O. (1950). Arterio-venous shunts in the human lung. *Proc. Soc. exp. Biol.* **75**, 827-829.



GOODALL —ARTERIO-VENOUS ANASTOMOSES IN THE SKIN OF THE CALF





- VASTARINI-CRESI, G. (1902). Comunicazioni dirette tra le arterie e le vene (anastomosi arterio-venose). Nota preliminare. *Monit. Zool. ital.* **13**, 136-142.
- WALDER, D. N. (1952). Arterio-venous anastomoses in the human stomach. *Clin. Sci.* **11**, 59-71.

# EXPLANATION OF PLATES

## PLATE 1

- Fig. 1. A contracted arterio-venous anastomosis in the perichondrium of the inner surface of a calf's ear, arising from a large artery (*A*) and opening into a small vein (*V*).
- Fig. 2. A convoluted arterio-venous anastomosis in the perichondrium of the inner surface of a calf's ear. The anastomotic vessel originates from an artery (*A*) and opens into a wide sack-like vessel, drained by three veins which finally join.
- Fig. 3. A map showing the distribution of arterio-venous anastomoses (circled in white) in a piece of perichondrium of a calf's ear. They occur usually near first or second branches of a main artery and are more numerous at the tip and periphery than in the centre.
- Fig. 4. A group of four arterio-venous anastomoses (*A.V.A.*) originating from the same artery.

## PLATE 2

- Fig. 5. An arterio-venous anastomosis (*A.V.A.*) in the skin of a calf's ear. The artery (*A*) and the vein (*V*) are in the second vascular plexus. The outlines of two sweat glands (*S.G.*) can be seen.
- Fig. 6. An arterio-venous anastomosis (*A.V.A.*) in the skin of a calf's forehead, originating from an artery (*A*) and opening into a vein (*V*). The outline of a sweat gland (*S.G.*) can be seen. The vessels are in the second vascular plexus.
- Fig. 7. An arterio-venous anastomosis (*A.V.A.*) in the second vascular plexus in the skin of the cheek of a calf, linking an artery (*A*) with a vein (*V*).
- Fig. 8. Cross sections of a small artery (*A*) and an arterio-venous anastomosis (*A.V.A.*) in the skin of the ear of a calf (stained Hx and eosin). The arterio-venous anastomosis can be recognized by the thickness of its wall and the large swollen nuclei of the 'epithelioid' cells of its media.

# THE ANATOMY AND FUNCTIONAL SIGNIFICANCE OF THE VASCULARIZATION OF THE ADRENAL GLAND IN THE RHESUS MONKEY (*MACACA MULATTA*)

BY R. G. HARRISON AND C. W. ASLING\*

*From the Department of Anatomy, University of Liverpool*

The vascularization of the adrenal gland in the rabbit, rat and cat (Harrison, 1951), although showing fundamental similarities to that in man (Gérard, 1913; Anson, Cauldwell, Pick & Beaton, 1947) has a simpler pattern. For example, although the rabbit adrenal is vascularized from the aorta, adrenolumbar and renal arteries, there are only about ten arteries approaching the periphery of the gland in contrast to the fifty or more arteries which supply the human adrenal. Individual adrenal arteries are end-arteries to the adrenal cortex of the rabbit, rat and cat (Harrison, 1951), but because of the profusion of arteries supplying the human adrenal gland, it is possible that this relationship may not hold for the adrenal gland of primates. It was therefore considered desirable to investigate the vascularization of the adrenal of the rhesus monkey with a view to determining the functional importance of individual adrenal arteries for the maintenance of adrenal cortical structure.

## MATERIAL AND METHODS

Two male and two female rhesus monkeys, varying in weight from 3.09 to 3.78 k.gm., were used for this study. The arterial supply of the adrenals in three monkeys was investigated by dissection, using a binocular microscope, following the injection of a 50 % suspension of barium sulphate (Micropaque; Damancy & Co. Ltd.) into the thoracic aorta. The average particle size of this medium is less than  $0.5\mu$ , and it has the advantage of filling the finest arteries although not passing through the capillary bed of the organ investigated; only the arterial system of the organ is therefore visualized. Radiography of the injected specimens was also performed, but the resultant arteriographs were difficult to interpret because of the complexity of the arterial pattern. The arteries supplying one adrenal of each monkey were injected immediately after killing the animal by means of an overdose of nembutal, the contralateral adrenal being used for experiments in which adrenal arteries were interrupted. In one monkey the intraglandular pattern of vessels was investigated following injection of indian ink into the thoracic aorta and subsequent sectioning of the adrenals and clearing of the sections. Coincident with the investigation of the arterial supply, operations were performed on the monkeys to interrupt arteries supplying the gland, using nembutal injected intraperitoneally as an anaesthetic, and approaching the adrenal through an incision in the hypochondrium just below and parallel to the costal margin. The animals were killed 5, 7 and 21 days after operation, and the operated adrenal examined histologically after staining with Heidenhain's iron haematoxylin and eosin.

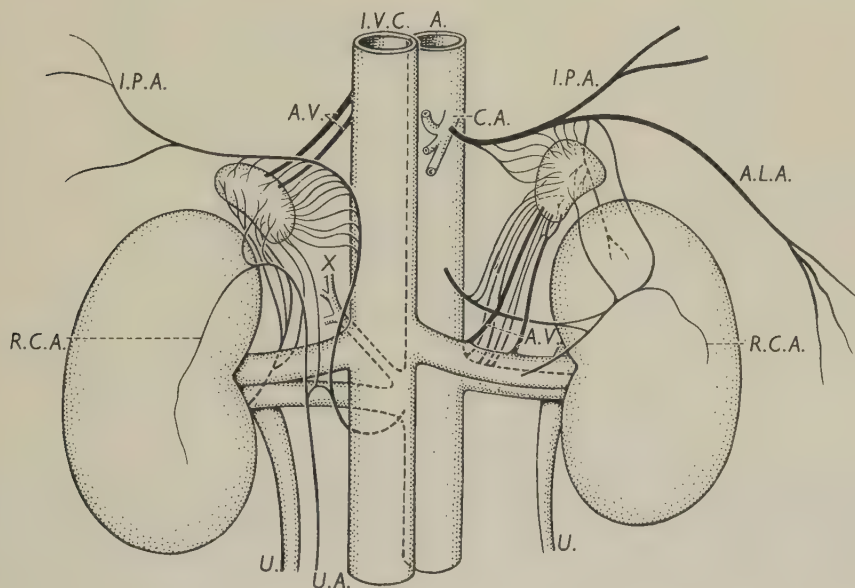
\* On leave from the Department of Anatomy, University of California under the auspices of a Fulbright Fellowship.

## RESULTS

### *The vascularization of the adrenal gland*

Both adrenals receive a large number of small arteries which form a rich network of vessels approaching the gland around its periphery. The two adrenals differ, however, in the number of arteries supplying them, and the sources of origin of the arteries (Text-figs. 1 and 2). On the left side the pattern is quite simple, the adrenal being supplied from three sources:

(1) A trunk arising from the coeliac artery. This supplies two or three arteries to the supero-medial aspect of the adrenal and then divides into an inferior phrenic and an adrenolumbar artery (Text-fig. 1). The adrenolumbar artery supplies four



Text-fig. 1. A drawing of the arterial supply of the adrenals of the rhesus monkey. The whole of the arterial supply to the left adrenal is shown, but only the superficial arterial supply to the right adrenal is depicted. The deep arterial supply arises from stem X shown in the figure, and its distribution is shown in Text-fig. 2. A., aorta; A.L.A., adrenolumbar artery; A.V., adrenal veins; C.A., coeliac artery; I.P.A., inferior phrenic artery; I.V.C., inferior vena cava; R.C.A., renal capsular artery; U., ureter; U.A., ureteric artery.

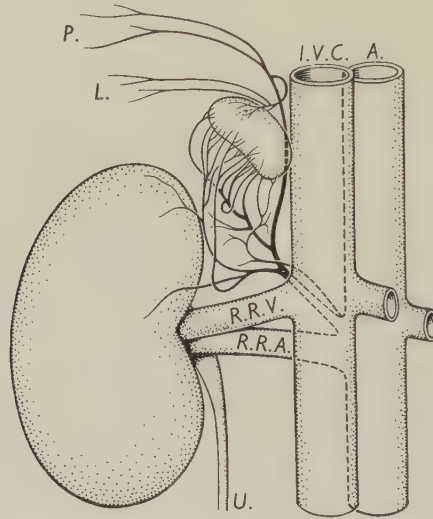
vessels to the anterior and posterior surfaces of the gland, one of these arteries passing on to the deep surface of the superior pole of the kidney as a renal capsular artery. A further renal capsular artery from the adrenolumbar artery supplying the anterior aspect of the kidney anastomoses with the termination of vessels arising from the aorta and renal artery.

(2) An artery arising from the aorta. This vessel furnishes two arteries to the medial aspect of the adrenal, and then passes on to the anterior aspect of the renal capsule to anastomose with the renal capsular vessels arising from the adrenolumbar and renal arteries.



(3) Four arteries arising from the left renal artery supply the infero-medial aspect of the gland.

The vascularization of the right adrenal is complicated by the fact that there is a well-marked superficial (anterior) and deep (posterior) supply of arteries to the gland. The superficial supply is derived from two vessels. One, arising from the aorta at the level of the right renal artery, passes posterior to the inferior vena cava, and follows a long course, at first anterior to the right renal vessels, and then in close contact with the right side of the inferior vena cava (Text-fig. 1) to end by arching over the superior aspect of the right adrenal, anterior to its two veins. This vessel furnishes twelve arteries to the medial and superior aspects of the gland and, at its



Text-fig. 2. The distribution of the deep arterial supply to the right adrenal. *A.*, aorta; *I.V.C.*, inferior vena cava; *L.*, lumbar arteries; *P.*, phrenic arteries; *R.R.A.*, right renal artery; *R.R.V.*, right renal vein; *U.*, ureter.

lower end gives off an arched vessel, lying anterior to the right renal artery, which provides an adrenal artery, and finally divides into a ureteric artery and a vessel which passes towards the lower pole of the adrenal to provide two further adrenal arteries and then finally recurve on to the anterior aspect of the renal capsule to anastomose with other renal capsular vessels. The other vessel composing the superficial arterial supply to the right adrenal arises from the deep aspect of the right renal artery at the hilum of the kidney and furnishes four or five arteries to the lower aspect of the adrenal.

The deep arterial supply to the right adrenal is provided from an arterial stem (*X* in Text-fig. 1) which arises from the angle between the aorta and right renal artery and then runs posterior to the inferior vena cava before dividing into three main vessels. The most superior of these passes posterior to the medial pole of the gland (Text-fig. 2), furnishes two adrenal vessels, and ends as inferior phrenic and lumbar vessels. This vessel corresponds to the adrenolumbar artery of the left side. The middle of the three vessels ends by giving five or six arteries to the adrenal,



network (Pl. 1, fig. 2), while others pass immediately into the cortex, without branching, as arteriae medullae (Pl. 1, fig. 4) to pass directly into medullary sinusoids without supplying the cortex.

#### *Experimental observations*

The arterial supply of the adrenals of the rhesus monkey is much more complex than in the case of lower mammals, and approximates more closely to the pattern found in man. In order to determine the functional significance of these arteries, operations were performed on the left adrenal in three monkeys, interrupting that adrenal artery which arises from the adrenolumbar artery, provides two vessels to the deep surface of the left adrenal, and then passes on to vascularize the renal capsule. Five days after this operation an area of focal necrosis of the adrenal cortex was found, involving all layers of the cortex, but sparing the immediately juxta-medullary cells of the zona reticularis (Pl. 1, fig. 5). The cells of the zona glomerulosa over most of the surface of the lesion are included in the necrosis, but those at the periphery are unaffected; in other words, the width of the necrotic area is greater in the depths of the lesion than at its surface. Within the necrotic area itself the nuclei of the cortical cells show karyolysis and karyorrhexis, the cells themselves losing their boundaries and their cytoplasm showing marked vacuolation with poor eosinophil staining. Seven days after operation the appearances of the area of necrosis are very similar, the centre of the lesion showing more pronounced necrosis (Pl. 1, fig. 6) and being more eosinophil than the remainder. The zona reticularis appears to be almost unaffected, however, and at one edge of the lesion the zona glomerulosa is quite intact. Twenty-one days after operation the area of focal necrosis is smaller (Pl. 1, fig. 7) and the cells within the lesion have lost all cytoplasmic staining, their nuclei showing pyknosis, karyolysis or karyorrhexis. The lesion involves only the zona fasciculata; the zona glomerulosa over the surface of the lesion, and the zona reticularis deep to it are intact and quite normal histologically. The zona glomerulosa is even thicker than normal over the surface of the lesion.

In a fourth monkey all adrenal arteries passing anterior to the right renal vessels were interrupted. Histological examination one week later revealed no damage at all in the cortex of the right adrenal.

#### DISCUSSION

The vascularization of the right adrenal in the rhesus monkey is clearly more complex than that of the left. There are more arteries entering the gland of the right side, and its vascularization from two sources produces a collateral circulation for at least part of it. Thus, if vessels crossing anterior to the right renal pedicle are interrupted, the anastomosis between renal capsular arteries from the superficial (Text-fig. 1) and deep (Text-fig. 2) arterial supplies ensures that no focal necrosis of the adrenal cortex should result. Interruption of individual adrenal arteries close to the gland, however, as shown by the experiments performed on the left side, produces an area of focal necrosis of the adrenal cortex. Arteries supplying the adrenal may therefore be considered as end-arteries to the cortex.

The appearance of the area of focal necrosis 21 days after operation suggests that



individual adrenal arteries are end-arteries only to the zona fasciculata of the cortex. This could only be reconciled with the involvement of the zona glomerulosa and zona reticularis in the lesion 5 and 7 days after operation either by a regeneration of cells of these two zones in the intervening period or by a variability of distribution to the adrenal cortex from the artery interrupted, which was identical in all cases. The former is unlikely in the case of the zona reticularis, since there was no evidence of mitosis in the cells of this zone; the thickness of the zona glomerulosa over the surface of the 21-day lesion was, however, greater than normal. Although no mitotic figures could be found in the zona glomerulosa over the lesion at any time period after operation, the appearance of the cells in this zone over the 21-day lesion is quite striking and different from the remainder of the zona glomerulosa in the same adrenal in that the nuclei of its component cells are flattened, disc-like and crowded upon each other to produce an appearance similar to the rouleau formation of erythrocytes (Pl. 1, fig. 8). This appearance could not have been produced by compression, since the lesion at 21 days would be contracting rather than expanding, but does suggest rapid formation of new cells. The other possibility, of a variable distribution of vessels to the adrenal cortex from the artery interrupted does, nevertheless, seem more likely, in view of the marked variability found in the arterial supply of the right adrenal.

Examination of the intraglandular distribution of vessels gives an explanation for the tendency of the focal necrosis to be restricted to the zona fasciculata. The capillary networks around cell groups in the zona glomerulosa anastomose with each other by way of the subcapsular arteries (Pl. 1, figs. 1 and 2). The adrenal cortex is, as it were, surrounded by a subcapsular arterial circle which gives off branches at intervals to the plexus of vessels in the zona glomerulosa. Similarly, the capillary sinusoids of the zona reticularis are wide, have rich interanastomoses and further anastomose by way of the connexions between medullary sinusoids. The radial vessels between the cell columns of the zona fasciculata, however, do not have such pronounced anastomoses, certainly not by vessels greater in calibre than themselves.

In the rhesus monkey as in other mammals already investigated (Harrison, 1951) the vessels of the adrenal cortex do not fill completely. There are areas of the cortex whose vessels do not fill at all. This patchy injection is due to several causes. Bennett & Kilham (1940) have claimed that the capillaries of the cortex can be narrowed by distension of the cells of the outer zona fasciculata with lipoid; but this would not explain the complete injection of sinusoids of the zona reticularis deep to an area of zona fasciculata which shows almost no filling of its vessels (Pl. 1, fig. 3), unless the zona reticularis vessels have been filled retrogradely by way of the anastomosis between medullary sinusoids. Although this is a possibility which cannot be ignored, another factor may be the wide calibre and complexity of the zona reticularis vessels forming a pool into which blood drains from zona fasciculata capillaries. By far the most important cause of diversion of blood from areas of the adrenal cortex, however, is the arteriae medullae; wherever such an artery is encountered in a section from an injected adrenal cortex, the area of cortex immediately surrounding it is almost empty of injection material (Pl. 1, fig. 4). These factors must be of great importance in the partitioning of blood flow through the adrenal cortex. For example, in an injection of a human adrenal with thorotrast through the adrenal vein,

the capillaries of the outer zona fasciculata and zona glomerulosa were poorly injected (Pl. 1, fig. 9) when examined subsequently by micro-radiography. Yet most investigations on the hormone output of the isolated adrenal gland are made on glands perfused through the adrenal vein; under these circumstances the perfusate would tend to lodge in zona reticularis sinusoids and pass out of the gland through arteriae medullae. At least the vascular mechanisms existing in the adrenal could ensure that not all parts or zones of the cortex, or even identical parts in different animals, would be irrigated by the perfusate, and this would account for quantitative and qualitative differences in the hormone content of the effluent in the perfusion of adrenal glands from similar or unrelated species. In the adrenal cortex of the bull, which is frequently used for perfusion studies, there are channels of wide diameter in the outer part of the zona fasciculata (Pl. 1, fig. 10), as well as arteriae medullae, which would ensure a diversion of blood from the capillary sinusoids of this part of the cortex.

#### SUMMARY

The arterial supply of the rhesus monkey adrenal is derived from the aorta, adrenolumbar and renal arteries, but the right adrenal has a more profuse vascularization than the left, being derived from superficial and deep arterial supplies, and having some thirty-eight arteries entering its periphery as against twenty arteries to the left adrenal. Variations can occur in the arterial supply of the right adrenal. Each adrenal is drained by two veins which pass into the inferior vena cava on the right and renal vein on the left. The intraglandular pattern of vessels is similar to that in other mammals, arteriae medullae also being present.

Interruption of individual adrenal arteries demonstrates that they are end-arteries to the adrenal cortex. The resultant area of focal necrosis tends to be restricted to the zona fasciculata.

The significance of the vascularization of the adrenal is discussed in relation to the blood supply of the human adrenal and the results of perfusion experiments.

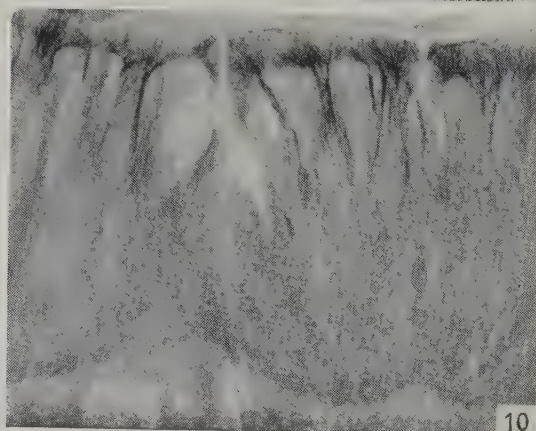
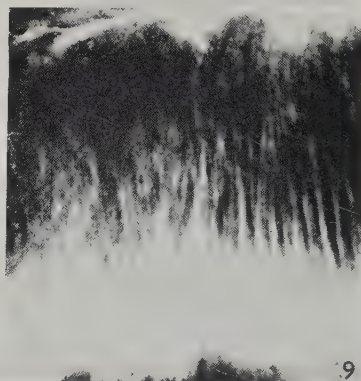
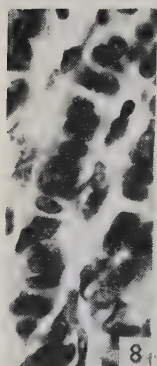
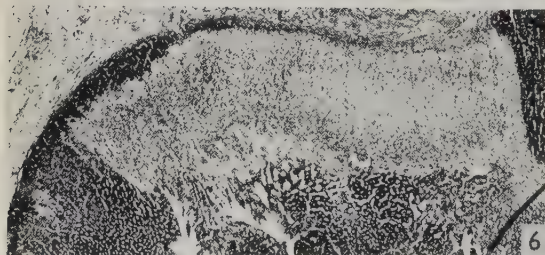
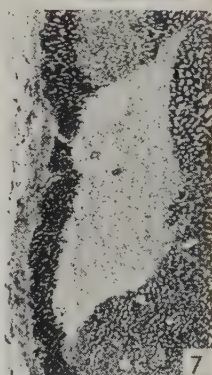
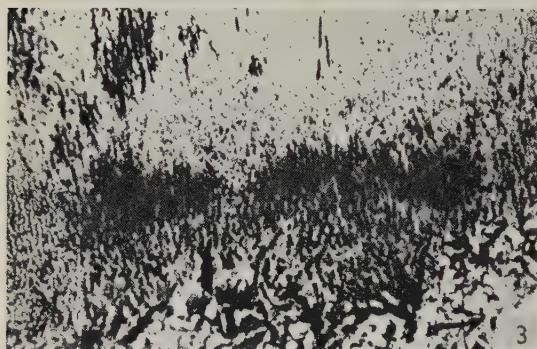
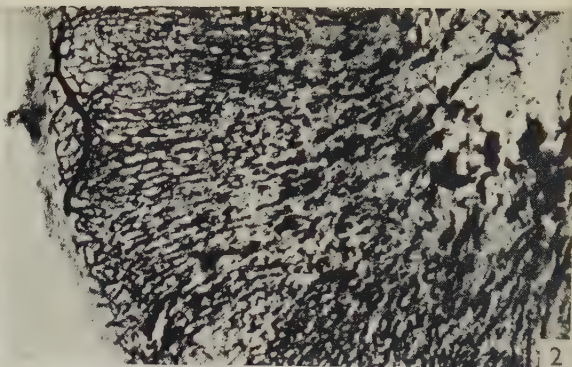
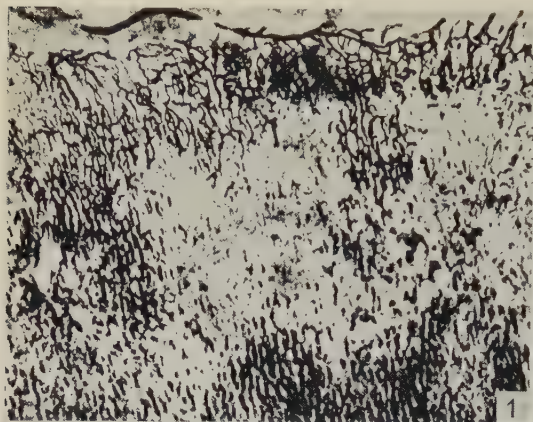
This research was aided by a grant from the Medical Research Council, and by a grant from the Dora Garrod Thomas Trust who provided the micro-radiographic apparatus (incorporating the Ehrenberg and Spear tube) used in the examination of the human and bull adrenals. We wish to thank Messrs L. G. Cooper and C. Fitz-Simon for their technical assistance.

#### REFERENCES

- ANSON, B. J., CAULDWELL, E. W., PICK, J. W. & BEATON, L. E. (1947). The blood supply of the kidney, suprarenal gland and associated structures. *Surg. Gynec. Obstet.* **84**, 313-320.
- BENNETT, H. S. & KILHAM, L. (1940). The blood vessels of the adrenal gland of the adult cat. *Anat. Rec.* **77**, 447-472.
- GÉRARD, G. (1913). Contribution à l'étude morphologique des artères des capsules surrénales de l'homme. *J. Anat., Paris*, **49**, 269-303.
- HARRISON, R. G. (1951). A comparative study of the vascularization of the adrenal gland in the rabbit, rat and cat. *J. Anat., Lond.* **85**, 12-23.







# EXPLANATION OF PLATE

Figs. 1-4 are photomicrographs of cleared sections,  $50\mu$  thick, of the adrenal cortex of the rhesus monkey following injection of indian ink into the arterial system of the adrenal. The magnification in each case is  $\times 50$ .

Fig. 1. The network of capillaries in the zona glomerulosa is clearly shown (upper part of the figure) fed at intervals along its length by a subcapsular artery. The capillary sinusoids of the outer zona fasciculata, in contrast to those in the inner part of the zona fasciculata (seen in the lower part of the figure), are poorly filled.

Fig. 2. The intracortical vessels in this specimen are well injected, and the sinusoids of the zona reticularis and medulla are clearly shown. A subcapsular vessel, on the left of the figure, dips down to the zona fasciculata and then recurves towards the capsule.

Fig. 3. The inner part of the adrenal cortex, showing the sinusoids of the zona reticularis and their drainage into medullary sinusoids.

Fig. 4. A segment of an arteria medullae in the adrenal cortex with an adjoining zone of poorly injected cortex.

Fig. 5. The zone of focal necrosis in the adrenal cortex five days after interruption of one of the adrenal arteries. Juxtamedullary cells of the zona reticularis are not destroyed, and the zona glomerulosa at the periphery of the lesion is unaffected.  $\times 17$ .

Fig. 6. The appearance of the area of focal necrosis seven days after interruption of an adrenal artery. Areas in the centre of the lesion show more pronounced necrosis. The zona reticularis (at the bottom of the figure) and the zona glomerulosa over the left-hand edge of the lesion are unaffected.  $\times 22$ .

Fig. 7. The area of focal necrosis 21 days after operation. The zona glomerulosa and z. reticularis are unaffected. The zona glomerulosa is thicker than normal.  $\times 25$ .

Fig. 8. A column of cells from the zona glomerulosa over the 21-day lesion, showing the flattened disc-like nuclei.  $\times 520$ .

Fig. 9. A microradiogram of a section,  $\frac{1}{8}$ -inch thick, of human adrenal cortex after injection of thorotrast into the adrenal vein. The vessels of the zona reticularis, in the lower part of the figure, are well filled with injection mass, while those in the outer zona fasciculata are poorly injected.  $\times 32$ .

Fig. 10. A microradiogram of a section,  $200\mu$  thick, of part of the adrenal cortex of a bull following injection of thorotrast into one of its arteries. The capillary network of the zona glomerulosa, and wide vascular channels in the outer part of the zona fasciculata, are clearly shown.  $\times 16$ .



# CONTRACTURE AND INTUSSUSCEPTIVE GROWTH IN THE HEALING OF EXTENSIVE WOUNDS IN MAMMALIAN SKIN

By R. E. BILLINGHAM\* AND P. B. MEDAWAR

*Department of Zoology, University College, University of London*

## INTRODUCTION

The purpose of this paper is to give evidence that intussusceptive growth may make an important contribution to the healing of extensive wounds in mammalian skin. It will be suggested that repair of the gaps left in the skin as a consequence of the destruction of the epidermis and the full thickness of the corium is brought about by two distinct processes: (a) contracture, a forced tissue movement that results in the closure of the wound by the apposition of its original edges; accompanied in its later stages by (b) a process of intussusceptive growth, a true enlargement of the skin, that goes some way towards making good the actual loss of skin substance. By 'intussusceptive growth' is meant the formation of new tissue upon or within the framework provided by pre-existing tissue. There is no evidence that the corium can regenerate *de novo*.

It is a corollary of this interpretation that the epithelialization of the wound, the building up of granulation tissue and its later conversion into white fibrous tissue are not definitive processes of healing and make no *substantive* contribution to the state of final repair. Migratory epithelium, granulation tissue and the product of its fibrous transformation are regarded as temporary organs of repair which disappear when their work is complete.

These generalizations define an extreme case, that in which contracture is demonstrably adequate to bring about the complete approximation of the original edges of the wound. They apply, therefore, only to the healing of wounds in a fully mobile integument. In areas where the skin is more firmly attached to the tissue beneath it (as in the greater part of human skin, and in the distal ear skin of, for example, rabbits and guinea-pigs) contracture cannot go to completion, so that a fibrous scar covered by epithelium of migratory origin remains. The same qualification presumably applies to wounds in a mobile integument which are too large to allow the complete apposition of the edges by contracture. The formation of even a stable scar can hardly be described as regeneration, for nothing has been regenerated: no true skin has formed anew; it must be regarded as a healing process which provides a stop-gap for the area that cannot be closed by the process of contraction. It is nevertheless our belief that healing of the kind we shall describe represents a norm from which the healing of human skin is an intelligible departure, and that the healing of extensive wounds in human skin can only be understood in the general context provided by an analysis of healing as it occurs in a fully mobile integument.

\* Research Fellow of the British Empire Cancer Campaign.



## METHODS

Contracture can of itself do no more than close a wound at the expense of mobile skin elsewhere on the body surface. Some other process is therefore presumably responsible for making good the loss of tissue substance. A process of intercalary or intussusceptive growth on the part of the remaining skin would normally be very difficult to reveal, because a large area of skin around the wound is subjected to the correspondingly weakened tensile forces produced by the process of contracture. There is, however, a simple way of concentrating the otherwise diffuse effects of chronic tension, i.e. to excise a raw area in such a way as to leave an island of undamaged skin in its centre (Pl. 1, fig. 1), or, alternatively, to complete the excision and then to place a skin graft in the centre of the wound (Pl. 2, fig. 9). As contracture proceeds, the central skin island or graft will be subjected to the same kind of chronic tension as the skin outside the wound. The power of the central island to respond to this chronic tension by mere elastic expansion is clearly limited by its small size; any further response to tension could therefore only take the form of an expansion by growth.

The expansion by growth of skin grafts in the situation just described was first recorded by Billingham, Krohn & Medawar (1951*a*), and it has been found by us in more recent work to be an invariable ingredient of the healing of large skin defects in laboratory mammals. Expansion by intussusceptive growth is a process entirely distinct from the enlargement of the epithelial surface of a graft by migratory outgrowth (as shown, for example, in Pl. 1, figs. 3, 7; Pl. 2, figs. 10, 12). Earlier investigators of wound healing in skin have described a process of enlargement in a stable fibrous scar left after wound contracture has come to a standstill (Carrel & Hartmann, 1916; Clark, 1919) but this, too, is a phenomenon of entirely different import, though it may have the same causal origin as the orderly intussusceptive growth of pre-existing skin.

The present work was designed to put our earlier observations upon a quantitative footing. The subjects of the experiments were three fully grown male rabbits weighing not less than 3 kg. In all three, a rectangular area of full-thickness skin (epidermis plus corium) was excised from the integument overlying the right-hand side of the chest; the incision was of such a depth as to expose, without damaging, the highly vascular planes of loose connective tissue which lie immediately superficial to the panniculus carnosus muscle (Pl. 1, fig. 1; Pl. 2, fig. 9).

In two of the rabbits (Exps. 1, 2) the excision was incomplete: a small rectangular island of skin was left behind in the middle of the wound bed (Pl. 1, fig. 1). In the third rabbit (Exp. 3) the entire rectangle of skin within the boundary incisions was removed, and a single disc of full-thickness skin was transplanted from the dorsum of the rabbit's ear to the middle of the raw area on its chest (Pl. 2, fig. 9). (Immediately before its removal the circumference of the graft had been accurately defined by a trephine 9 mm. in diameter.)

The operations were conducted and dressings were applied in the way described in full by Billingham & Medawar (1951). The only initial difference between Exps. 1, 2 and 3 was that the latter made use of a free skin graft and the former of a residual skin island with its underlying attachments undisturbed. In the outcome, the results of the three experiments were virtually indistinguishable.

After 75-79 days, when contraction of the wound and expansion of the central skin were complete, a second-stage operation was carried out in order to exaggerate the consequences of the first: a new rectangular area of skin was excised in such a way as to leave the original (but now expanded) island or graft in position. (The second raw area had been intended to be a replica of the first, but the effect of the gaping referred to below was to make it, in Exps. 1 and 2, about twice as large.) The healing process was again followed to completion.

Direct measurements supplemented by photographs at constant magnification were made at suitably spaced intervals during and after the closure of the wound. The principal measurements (taken to the nearest 0.5 mm. with dividers while the animal was in full relaxation under 'nembutal' anaesthesia) were (a) of the distances between the original skin edges measured across the wound bed, and (b) of the linear dimensions of the graft or central island. All measurements were taken from true skin edges and never from the advancing edge of migratory skin epithelium. The true skin edge was clearly enough defined by the abrupt disappearance of the typical collagenous pattern of the corium and of the openings of the hair follicles.

#### OBSERVATIONS

Measurements representing the progress of wound contracture and graft expansion in the three rabbits (Exps. 1-3) are set out in Tables 1-3 and Text-figs. 1-3 respectively. Selections from the serial photographs of healing in two of the three rabbits (Exps. 2, 3) appear in Pls. 1 and 2.

Table 1. *Contraction of wound bed and expansion of central skin island in Exp. 1*

Areas are expressed in sq.cm. See also Text-fig. 1.

Day after		Area of		Notes
First-stage operation	Second-stage operation	Wound bed	Skin island	
0	—	{ 30.25 41.40	{ 2.25 1.05	Area defined by incisions After excision of skin
6	—	30.25	1.20	—
10	—	15.55	1.20	—
14	—	11.90	1.70	—
20	—	7.75	1.95	—
27	—	6.40	2.40	—
42	—	—	3.95	—
48	—	—	4.80	—
79	0	{ — 79.20	{ 6.95 3.15	Before 2nd-stage excision After 2nd-stage excision
90	11	33.00	2.95	—
104	25	15.00	7.40	—
123	44	—	7.85	—
142	63	—	11.40	—

As the first entries in each Table show, the central skin graft or island contracted immediately the excision released it from the prevailing tension of the skin; correspondingly, the wound bed gaped, so increasing the area bounded by the original scalpel incisions by as much as 50 %.

The general wound bed contracted at first at a precipitous rate (Text-figs. 1-3) and then progressively more slowly. The grafts expanded very little during the

Table 2. *Contraction of wound bed and expansion of central skin island in Exp. 2*

Areas are expressed in sq.cm. See also Text-fig. 2 and Pl. 1.

Day after		Area of		Notes
First-stage operation	Second-stage operation	Wound bed	Skin island	
0	—	{ 30.25	{ 2.25	Area defined by incisions After excision of skin
6	—	{ 44.20	{ 1.30	
10	—	35.10	1.75	—
14	—	28.60	1.95	—
20	—	18.90	2.00	—
27	—	13.80	2.55	—
42	—	> 6.50	3.10	—
48	—	—	3.50	—
79	0	—	4.95	—
90	11	{ —	{ 5.75	Before 2nd-stage excision After 2nd-stage excision
104	25	{ 83.65	{ 3.50	
123	44	38.95	2.85	—
142	63	18.15	5.75	—
		—	10.05	—
		—	12.70	—

Table 3. *Contraction of wound bed and expansion of central skin graft in Exp. 3*

Areas are expressed in sq.cm. See also Text-fig. 3 and Pl. 2.

Day after		Area of		Notes
First-stage operation	Second-stage operation	Wound bed	Skin graft	
0	—	{ —	{ 0.65	Area before excision Area after excision
11	—	{ 54.60	{ 0.45	
15	—	35.20	—	—
25	—	28.35	0.65	—
44	—	17.95	0.80	—
63	—	—	2.45	—
75	0	—	3.70	—
86	11	{ —	{ 4.05	Before 2nd-stage excision After 2nd-stage excision
111	36	{ 56.15	{ 3.70	
133	58	39.00	3.40	—
		22.80	5.65	—
		—	6.00	—

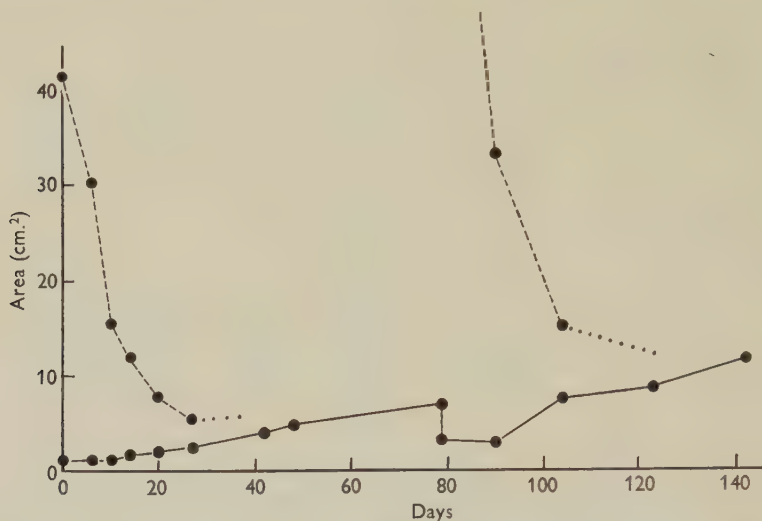
earlier and very rapid phase of contracture, but more rapidly, though at an approximately constant rate, during the later stages. Graft expansion therefore presumably represents the response to chronic, not merely to acute tension.

The central skin islands contracted for a second time immediately the second-stage excision of the surrounding skin released them from tension. Contraction continued during the first 10–15 days after the second-stage operation, in spite of the rapid approximation of the outer edges of the wounds. Each island then expanded far beyond the area which it had achieved at the end of the first stage of healing.

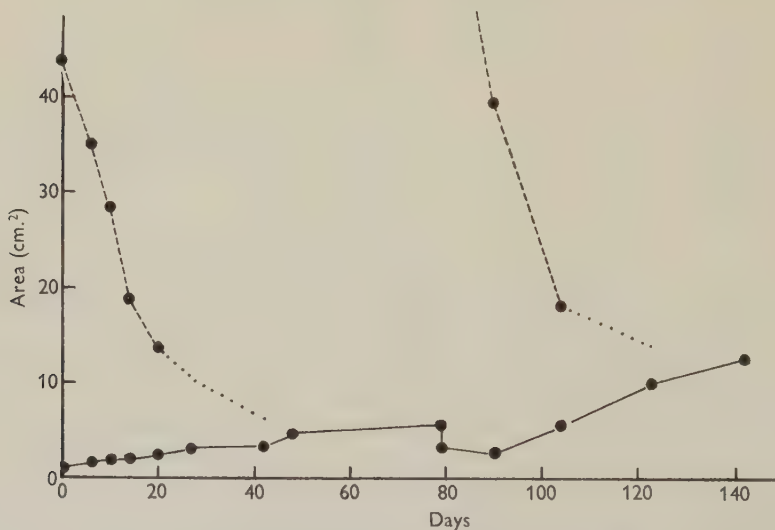
The wound gaped widely when the skin was excised at the second-stage operation. The contracture of the central island and the gaping of the wound bed at the second-stage operation make it clear that wound closure is brought about by a ‘pull’ from within the wound bed that draws and holds the skin edges together and not to a force directed inwards from the surrounding skin or outwards from the expanding graft (Lindquist, 1946).



During the expansion of the central skin island each cluster of hair follicles became separated from its neighbours by a distance roughly proportional to the linear



Text-fig. 1. Exp. 1 (see also Table 1). Illustrating the contraction of the wound bed and the compensatory expansion of the central skin island contained within it. The broken line represents the total area contained within the outer perimeter of the wound (i.e. central island plus wound bed); the solid line represents the area of the central island. The second-stage operation was carried out at the 79th day.

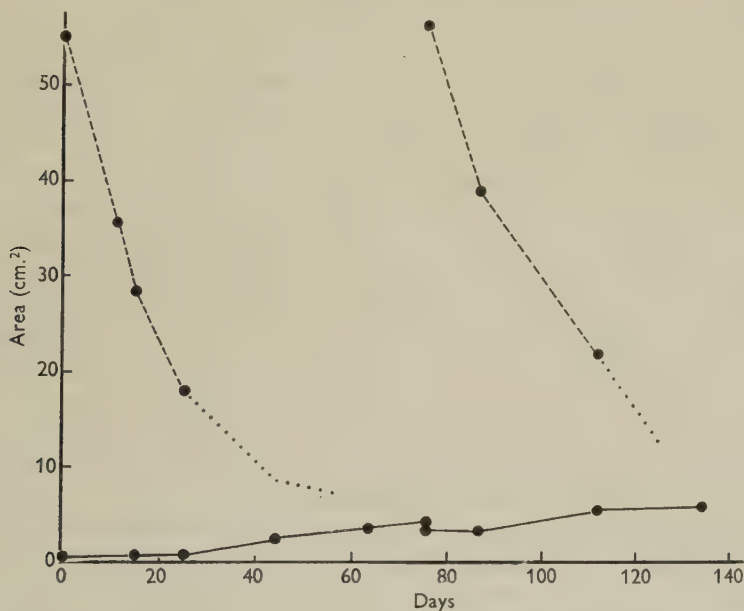


Text-fig. 2. Exp. 2 (see also Table 2). For explanation, see legend to Text-fig. 1.

enlargement of the graft. No *new* hair follicle clusters were formed; the basic collagenous pattern of the corium remained unchanged. Histological examination (Pl. 2, fig. 14) showed that new collagen had been formed in relationship to the pre-

existing fibrous structure, for the grafts retained their normal thickness and there was no evidence of fibrous attenuation. The process of graft expansion is not therefore one in which the graft takes a purely passive part. It involves the orderly new formation of dermal connective tissue in relation to the pre-existing framework.

The end of the healing process is shown in Pl. 1, fig. 8 and Pl. 2, fig. 13, from which it can be seen that the original true skin edges of the central skin island and the boundary of the excised wound became closely and exactly apposed to each other. All the migratory epithelium that originally grew outwards from the central island



Text-fig. 3. Exp. 3 (see also Table 3). As Text-figs. 1, 2 except that the central skin island began as a free ear-skin graft, and the second-stage operation was carried out on the 75th day.

and inwards from the edges of the wound simply disappeared. The 'false skin' composed of migratory skin epithelium underlain by fibrous tissue is therefore an ephemeral structure which makes no substantive contribution to the state of final repair.

Some reference should be made to the geometrical pattern of contracture. An empty rectangular raw area does not keep its shape during contracture: the sides cave in convexly and the corners end, as they begin, farthest from the middle (see the figures of Billingham & Reynolds, 1952). Although this pattern is modified by the presence of a central patch of skin, it is clear that the same principle applies, for the final lines of apposition radiate as spokes from the centre and the skin edge at the original corners of the raw area is that which stands farthest from the central area of normal skin (Pl. 1, figs. 5, 6, 8; Pl. 2, figs. 11, 13). The work of Abercrombie, Flint & James (1954) on the healing of full-thickness skin defects in rats has shown that the total length of the perimeter of a raw area may diminish by only about 25 % during contracture, so that the final pattern of the lines of apposition is pro-

bably one of the simplest that could result from a moderate reduction of the length of the wound perimeter accompanied by a symmetrical obliteration of the space contained within it.

#### INTERPRETATION

The evidence presented above shows that an extensive defect in the integument of a rabbit's trunk heals by a twofold process: (a) contracture, which closes the wound, and (b) a generalized intussusceptive growth of the residual skin, which goes some way towards making good the original loss of substance.

There is no reason to doubt that the expansion of skin is made possible by the readjustment and growth of the fibrous endoskeleton of the corium in response to chronic tension. It is well known that fibroblast cells adopt an orientation that conforms to the lines of superimposed tensions (Weiss, 1929, 1933) and that the growth of many kinds of mesenchymal matrix is enhanced by mechanical stimulation (for tendon, see Levy, 1904; for bone, Glücksmann, 1942). Our findings thus extend to skin a principle known to obtain in the growth and repair of other collagenous tissues. It is very likely that the expansion which we have caused to occur during the regeneration of the integument is identical in its mechanism to that which occurs in the ordinary growth in area of the skin during later development, for we find that skin grafts transplanted from adult to newborn or juvenile animals expand with the growth of their hosts in just the manner of the skin islands described here.

To describe migratory epithelium and granulation tissue and its fibrous derivatives as 'temporary organs of repair' (see Introduction, p. 114) is in no sense a depreciation of their importance. Migratory epithelium fulfils at least three functions. First, it undermines and causes the sloughing away of dead superficial tissue (see Medawar, 1944, figs. 38, 39) and scabs. (The formation of a scab must be regarded as a normal accompaniment of healing; its failure to form in a covered sterile wound is an artefact of surgical care. The persistence of a scab could obviously be a serious impediment to healing.) Secondly, the presence of an epithelial cover expedites the fibrous transformation of granulation tissue—a property analysed with great care by Gillman, Penn, Bronks & Roux (1953), and one which is particularly conspicuous in wounds in which collagenization has been retarded by the administration of cortisone (Billingham *et al.* 1951*a*, *b*). Thirdly, it stops the seepage of body fluids through the surface of the wound and reduces the risk of infection. But it is most important to notice that a property which migratory epithelium manifestly does *not* possess is that of preventing wound contracture (see Billingham & Reynolds, 1952). (If indeed it promotes the collagenization of the wound bed, and if this wound collagen has the function attributed to it below, it should follow that epithelialization hastens contracture—and with contracture the migratory epithelium disappears.)

It has been well argued by Lindquist (1946) that the function of the fibrous tissue enclosed within the perimeter of the wound is to provide the tensile forces that are responsible for contracture. Whether the tensile force is due to a shortening of collagen fibres during their maturation, to a kinking of the fibres, or to a contraction of the cells that make up the rich mesenchymal population of young fibrous tissue (a possibility suggested to us by Mr M. Abercrombie) has yet to be determined.



#### REPAIR IN HUMAN SKIN

There is no reason to doubt that our inferences, although drawn from experiments on the trunk skin of rabbits, apply with equal force to wounds of the same relative size in equally mobile areas of the integument in other mammals. They do not apply to human beings. The greater part of the integument of the great majority of mammals is rather loosely attached to and consequently freely mobile upon the body wall—a state of affairs doubtless correlated with the presence of a substantial layer of cutaneous striped muscle, the panniculus carnosus, which endows mammals with the power to twitch their skins. Contracture does not entail disablement: the skin has plenty of ‘give’. In human beings the panniculus carnosus is vestigial and the skin is much more closely knit to the tissues beneath it. Contracture in human beings, so far from being an efficient instrument of repair, may therefore lead to constriction, distortion, immobilization and other disabilities. Human beings may be said to have retained the mechanism of healing by contracture but to have lost the anatomical prerequisites which enable it to proceed to good effect. It is to be doubted that any efficient ‘natural’ process of wound closure exists in human skin. Much the same is true of the healing of full-thickness defects in the skin of the distal parts of the ears of laboratory mammals (e.g. rabbits, guinea-pigs) where the skin is closely knit to the subcutaneous tissues. In this situation, as in men, contracture leads to a deformation and gathering of the surrounding skin and the residual gap that cannot be closed by contracture is occupied by a stable fibrous scar covered by skin epithelium of migratory origin.

In clinical practice, extensive gaps in human skin are made good by artificial replacement, i.e. by grafting, an act which forestalls and circumvents contracture, the ‘natural’ process of repair. If our reasoning is correct, complete skin replacement by grafting is the only theoretically sound method of repairing full-thickness skin defects in human beings. Inasmuch as the use of skin *autografts* is at present obligatory for permanent repair, such an operation is not always possible; few problems in plastic surgery can be more insistent and of longer standing than that of repairing wounds in human skin that are too large to be covered in their entirety by grafts of the patient’s own skin. Our interpretation of the purely temporary function of migratory skin epithelium suggests that to attempt to repair such wounds by covering them with epithelium alone is as unsound in theory as it has proved to be inefficacious in practice (Billingham & Reynolds, 1952; Medawar, 1954). The accepted solution of the problem is therefore ‘patch grafting’—the use of whatever autogenous skin may be available as an interrupted patchwork of grafts cut into strips or into rectangles of about the size of postage stamps.

One important advantage of patch grafting over the use of the available skin as a single undivided sheet is that patch grafting converts a single large wound into what is, in effect, a large number of proportionately smaller wounds which heal concurrently, each faster than a single large wound. Our observations on the expansion of skin grafts in rabbits point to another possible theoretical advantage of patch grafting in human beings over other available makeshifts: the tension generated by contracture of the uncovered areas of the wound may cause the patches to grow by expansion, so that the patch graft is ultimately worth more than its original area of

sound skin. It is therefore to be hoped that surgeons will investigate the growth of patch grafts in human beings.

#### SUMMARY

1. The closure of extensive full-thickness skin defects in rabbits is accomplished by contracture, a forced movement of the integument that results in the closure of the wound by the apposition of its original edges.

2. A skin graft or skin island lying within a raw area undergoing contracture itself undergoes a compensatory expansion produced by a true intercalary or intussusceptive growth. It is inferred that while contracture brings about the closure of a skin wound, the loss of skin substance is at least partly made good by a true growth of the remaining skin.

3. It is argued that the covering of the wound by migratory skin epithelium and the fibrous transformation of granulation tissue in the wound bed are not definitive processes of healing and make no substantive contribution to the state of final repair. The migratory epithelium serves to undermine dead superficial tissue and scab, to arrest the loss of body fluids, and to expedite the collagenization of the wound bed. The fibrous tissue itself is thought to be the origin of the tensile forces responsible for contracture of the wound bed and growth of the surrounding skin.

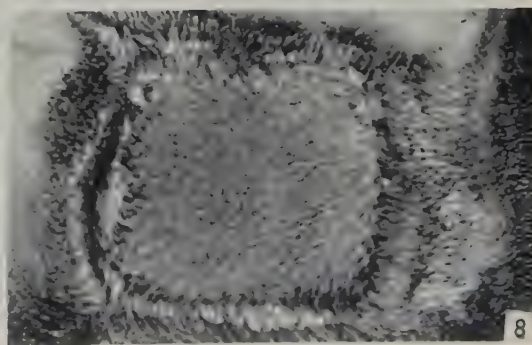
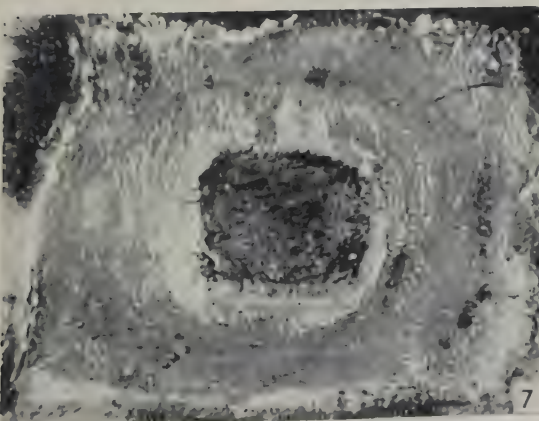
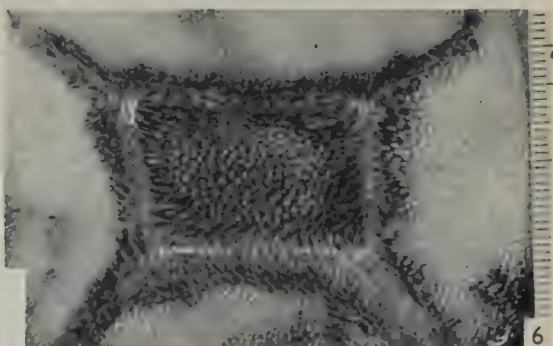
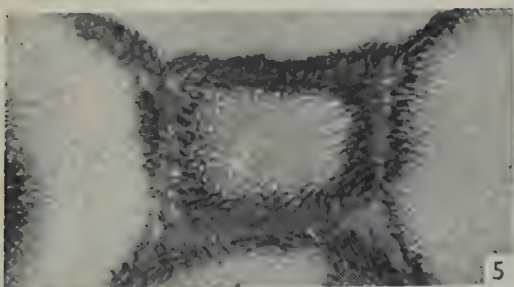
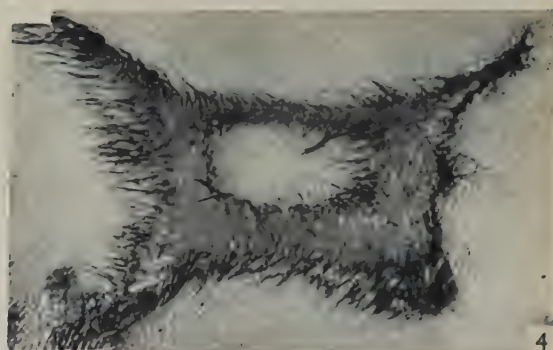
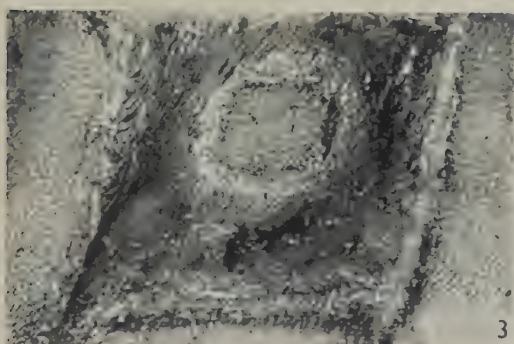
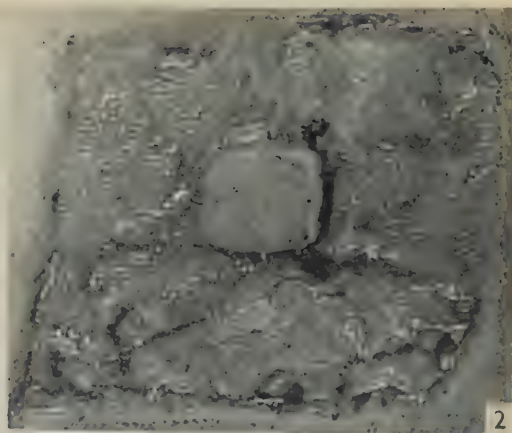
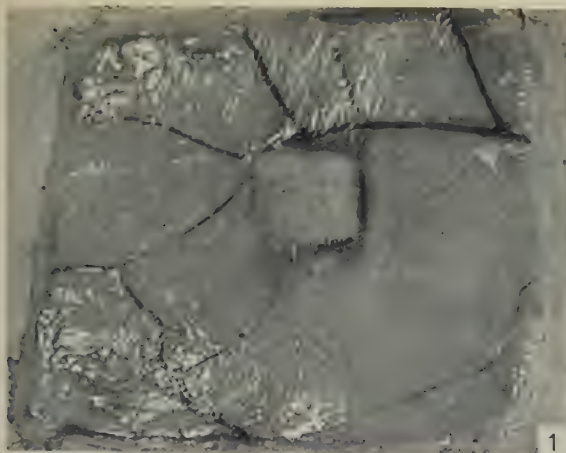
4. For what are believed to be primarily anatomical reasons, human beings are an exception to the rule that contracture is an efficient method of wound closure, and it is argued that skin replacement is the only theoretically defensible method of attempting to achieve the efficient repair of the skin.

We are particularly grateful to Mr M. Abercrombie for his criticisms of this manuscript. We are obliged to Mr T. H. Courtenay for his surgical assistance and to Mr W. Brackenbury for taking the photographs. Some of the costs of this work were defrayed by a grant from the Nuffield Department of Plastic Surgery, University of Oxford.

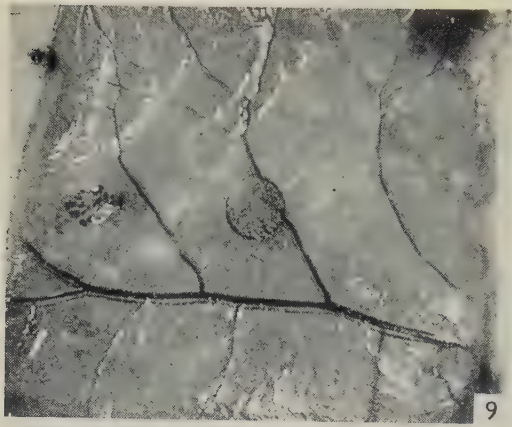
#### REFERENCES

- ABERCROMBIE, M., FLINT, M. H. & JAMES, D. W. (1954). Collagen formation and wound contraction during repair of small excised wounds in the skin of rats. *J. Embr. exp. Morph.* **2**, 264-274.
- BILLINGHAM, R. E., KROHN, P. L. & MEDAWAR, P. B. (1951*a*). Effect of cortisone on survival of skin homografts in rabbits. *Brit. med. J.* **1**, 1157-1163.
- BILLINGHAM, R. E., KROHN, P. L. & MEDAWAR, P. B. (1951*b*). Effect of locally applied cortisone acetate on survival of skin homografts in rabbits. *Brit. med. J.* **11**, 1049-1052.
- BILLINGHAM, R. E. & MEDAWAR, P. B. (1951). The technique of free skin grafting in mammals. *Brit. J. exp. Biol.* **28**, 385-402.
- BILLINGHAM, R. E. & REYNOLDS, J. (1952). Transplantation studies on sheets of pure epidermal epithelium and on epidermal cell suspensions. *Brit. J. plast. Surg.* **5**, 25-36.
- CARREL, A. & HARTMANN, A. (1916). Cicatrization of wounds. I. The relation between the size of a wound and the rate of its cicatrization. *J. exp. Med.* **24**, 429-450.
- CLARK, A. H. (1919). The effect of diet on the healing of wounds. *Bull. Johns Hopk. Hosp.* **30**, 117-120.
- GILLMAN, T., PENN, J., BRONKS, D. & ROUX, M. (1953). Reactions of healing wounds and granulation tissue in man to auto-Thiersch, autodermal and homodermal grafts. *Brit. J. plast. Surg.* **6**, 153-223.
- GLÜCKSMANN, A. (1942). The role of mechanical stresses in bone formation *in vitro*. *J. Anat., Lond.*, **76**, 231-239.
- LEVY, O. (1904). Über die Einfluss von Zug auf die Bildung faserigen Bindegewebes. *Arch. EntwMech. Org.* **18**, 184-246.

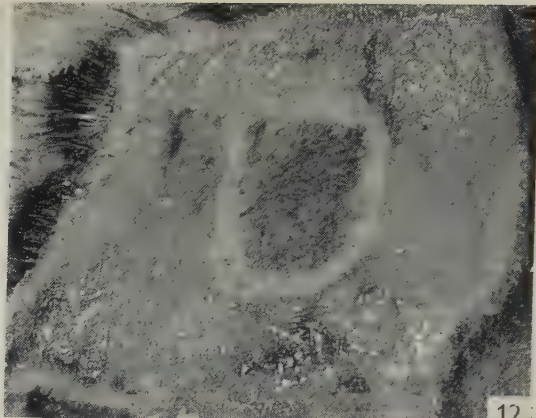




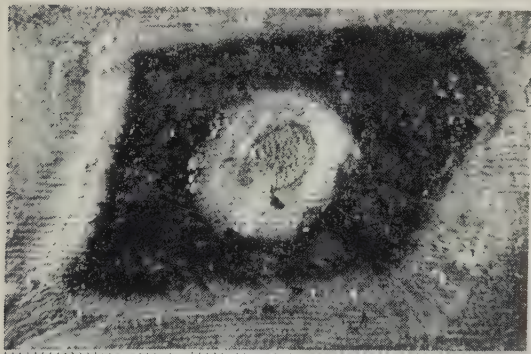




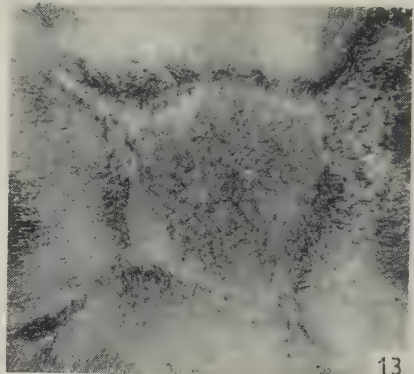
9



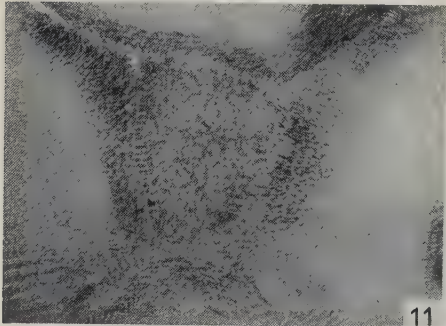
12



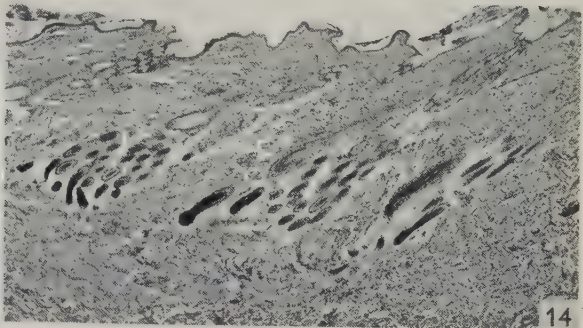
10



13



11



14

- LINDQUIST, G. (1946). The healing of skin defects. An experimental study on the white rat. *Acta Chir. Scan.* **94**, suppl. 107, p. 163.
- MEDAWAR, P. B. (1944). The behaviour and fate of skin autografts and skin homografts in rabbits. *J. Anat., Lond.*, **78**, 176–199.
- MEDAWAR, P. B. (1954). The storage of living skin. *Proc. Roy. Soc. Med.* **47**, 62–64.
- WEISS, P. (1929). Erzwingung elementärer Strukturverschiedenheiten am *in vitro* wachsenden Gewebe. *Roux Arch. EntwMech. Org.* **116**, 438–554.
- WEISS, P. (1933). Functional adaptation and the role of ground substances in development. *Amer. Nat.* **67**, 322–340.

## EXPLANATION OF PLATES

### PLATE 1

Experiment 2 (see also Table 2 and Text-fig. 2). Illustrating the contraction of the wound bed and the expansion of a skin island contained within it after the first-stage (figs. 1–6) and second-stage (figs. 7, 8) operations. All photographs were taken at the magnification indicated by the millimetre scale on fig. 6.

- Fig. 1. Day 0. The operation field after excision of the skin in such a way as to leave a rectangular area undisturbed in the centre. Note the prominent blood vessels running in a plane superficial to the panniculus carnosus muscle.
- Fig. 2. Day 6. Slight contracture of the wound bed; building up of granulation tissue. Epithelial ingrowth from edges of the wound and outgrowth from the central island is just beginning.
- Fig. 3. Day 14. Further contracture and epithelial outgrowth. Almost the whole surface of the wound bed is now epithelialized.
- Fig. 4. Day 27. Showing the characteristic 'caving in' of the wound edges as contracture proceeds. Hairs are growing from the margins of the central island and the perimeter of the wound bed. The wound bed is fully epithelialized.
- Fig. 5. Day 42. The wound bed has now been almost completely obliterated as contracture approaches completion. Note expansion of the graft.
- Fig. 6. Day 48. Contracture is almost complete: the epithelium that formed the original surface of the wound bed has almost completely disappeared. Note the great enlargement of the central island and the spacing out of the clusters of hair follicles.
- Fig. 7. Day 79 + 15. Fifteen days after the second-stage excision at 79 days (see text), illustrating a stage equivalent to that illustrated in fig. 3.
- Fig. 8. Day 79 + 62. Completion of healing. Compare the size of the central island with its size in fig. 1, and the wide spacing apart of clusters of hair follicles. No migratory epithelium remains. See Pl. 2, fig. 14.

### PLATE 2

Experiment 3 (see also Table 3 and Text-fig. 3). Illustrating the contraction of the wound bed and the expansion of an ear-skin graft contained within it after the first-stage (figs. 9–11) and second-stage (figs. 12–13) operations. All photographs were taken at the magnification indicated by the millimetre scale on fig. 10.

- Fig. 9. Day 0. A circular disc of full-thickness dorsal ear skin has been transplanted to the centre of an extensive raw area prepared in the skin of the chest. Compare Pl. 1, fig. 1.
- Fig. 10. Day 15. Contracture of the raw area; outgrowth of epithelium from the graft and ingrowth from the perimeter of the wound bed.
- Fig. 11. Day 62. Completion of healing after the first-stage operation. Note the pattern of contracture of the raw area, and the disappearance of epithelium originally contained within its margins. Compare the present size of the graft with its original size (fig. 9).
- Fig. 12. Day 75 + 11. Eleven days after the second-stage operation carried out on day 75, a stage essentially comparable with that illustrated by fig. 10, though the central graft is now much larger.
- Fig. 13. Day 75 + 58. Completion of healing; approximation of the original edges of the wound. Note the great expansion of the graft and the sparseness of the hair pelt caused by the separation of the hair follicles during intercalary growth.
- Fig. 14. Exp. 2, Day 79 + 62. A transverse section of the graft illustrated by Pl. 1, fig. 8. Note that the corium has not thinned during expansion, but retains the normal pattern of mature collagen fibres. Ehrlich's haematoxylin and eosin.  $\times 20$ .



# MITOTIC ACTIVITY IN THE VAGINAL EPITHELIUM OF THE MOUSE FOLLOWING LOCAL OESTROGENIC STIMULATION

By J. D. BIGGERS AND P. J. CLARINGBOLD

*Department of Veterinary Physiology, University of Sydney, N.S.W., Australia*

The mitotic activity in the vaginal epithelium of the ovariectomized mouse, following the subcutaneous injection of approximately  $5\mu\text{g}$ . oestrone, was studied by Allen, Smith & Gardner (1937) with the colchicine technique. These workers used only a few animals and concluded that cell division commences in less than  $9\frac{1}{2}$  hr. from the time of injection, reaches a maximum rate at 37 hr. and declines by 48 hr.

Several workers have been interested in the mitogenic action of oestrogens in other tissues. Bullough (1950, 1952) studied the epidermis of the ear of the mouse and suggested that oestrone has a dual action (i) by producing hyperglycaemia, and (ii) by direct cellular action. Hamm & Cappel (1940) studied the effect of oestrone on fibroblasts, from mouse embryo heart, in tissue culture and found no effect. In all this work the tissues studied are not those having a high competence to react to oestrogens, and thus they may not be the best material in which to study the direct action of the hormone.

Oestrogens may act directly on the epithelium of the vagina which has been separated from its blood supply. This has been clearly established by tissue culture experiments in the guinea-pig (Coujard, 1943), in the mouse (Hardy, Biggers & Claringbold, 1953) and in the rat (Kahn, 1954). The experiments of Robson & Adler (1940) and Emmens (1942) with mice having double vaginal sacs have shown that small doses of true oestrogen administered intravaginally are local in their action, and presumably do not elicit systemic responses. The intravaginal route of administration of oestrogens therefore provides a means of studying mitotic activity produced by direct oestrogen action, and also distinguishes between the two methods of action proposed by Bullough (1950). The only comparable work on the local action of oestrogens on mitosis is that of Stein & Allen (1942) who injected oestrone into the ovarian bursa of the mouse, and observed an increase in the mitoses of the germinal epithelium of that ovary 30 hr. later. The other ovary was affected but to a lesser degree. Bullough (1942) claims to have demonstrated a similar local phenomenon in the mouse following intraperitoneal injection of oestrogens into 'the vicinity of the ovary'. This evidence is unacceptable, however, since the possibility of absorption into the blood stream is not eliminated by this technique.

In this paper the mitotic activity in the vaginal epithelium of the ovariectomized mouse, following the intravaginal administration of oestrone, is described.

## MATERIAL AND METHODS

Fifty ovariectomized albino mice have been used in this study. One week before use they were primed by the subcutaneous injection of  $1\mu\text{g}$ . oestrone in peanut oil. Individual animals were allotted to the experimental groups with tables of random numbers.



Each animal was given  $6.4 \times 10^{-3}$   $\mu$ g. oestrone in one intravaginal injection of 0.01 ml. of 1% aqueous egg albumin solution. When administered in this way the dose of oestrone is expected to produce vaginal cornification in all animals, the effect of the protein being to retain the oestrone at its site of action (Biggers, 1951, 1953*a*; Biggers & Claringbold, 1954). Ten groups, each of five animals, were used and a group was killed every 6 hr., from 0 to 54 hr. inclusive after the injection of oestrone. 9 hr. before each animal was killed 0.1 mg. colchicine in 0.05 ml. distilled water was injected subcutaneously, in order to arrest mitoses.

The action of colchicine has been reviewed recently by Levine (1951). Following injection of the alkaloid 'the nuclear membrane disappears, the cytoplasm becomes turgid, the chromosomes agglutinate, and mitosis is arrested in metaphase'. By counting the mitoses accumulated over a given time an estimate may be made of the mitotic rate, although this estimate is not necessarily the absolute number of mitoses present in the organ (cf. Brues, 1951). Since in this study the number of mitoses accumulated over a fixed period are compared, a relative estimate is all that is required and therefore the above method is considered satisfactory. Further, unlike Bullough (1950) we have made no attempt to estimate duration of mitosis.

The vagina was removed, fixed in Gendre's fixative and embedded in wax. Transverse sections  $7\mu$  thick were stained in Mayer's haematoxylin and eosin.

Two types of observation have been made in order to place the results on a quantitative basis (i) the number of arrested mitoses in a given length of epithelium, (ii) the number of cell layers. Sections were taken at random from the organ so as to obtain an overall picture of the vaginal response, and therefore any findings in this work are applicable to the vaginal epithelium as a whole rather than to an isolated level chosen for study.

(i) *Arrested mitoses.* For each animal at least five sections were available for examination. All observations were made with a binocular microscope with  $7\times$  eyepieces and a 1/6th-inch objective (total magnification  $420\times$ ). Five fields were selected at random from the sections, care being taken not to use the same field more than once, and with the restriction that the epithelium lay across a diameter of the field. The total number of arrested mitoses were counted in each field and the final score for each animal was the sum of five counts.

(ii) *Cell layers.* Five regions of the epithelium were selected at random from the same sections used above, and the number of cell layers counted. The average of these five observations was the score allotted to each animal. In the animals where stratification and cornification had occurred the exact number of layers was difficult to assess, and therefore the number observed may be underestimated.

Since the changes in the epithelium are pronounced, it has been considered unnecessary to use more complicated measures to describe the epithelial response. The statistical analysis of these observations will be described together with the results.

## RESULTS

### *Quantitative observations*

(i) *Arrested mitoses.* The observations are given in Table 1. It is obvious from these figures that increased variability is associated with large numbers of arrested mitoses. When the variability depends on the level of response, the use of the analysis

of variance in the estimation of regression coefficients is invalid (Cochran, 1947). A transformation defined by  $Y_1 = \log_{10}(Z_1 + 1)$  was applied to the data of Table 1, where  $Z_1$  is the number of arrested mitoses. Unity was added to the values of  $Z_1$  to remove zero values. Bartlett's test for homogeneity of variances was applied (Rao, 1952), and this gave, using the second approximation,  $\chi^2_{(9)} = 19.5$ ,  $0.05 > P > 0.02$ . Inspection of the transformed data indicates that the borderline significance of this  $\chi^2$  value is due to large variability in the regions of rapid change of mitotic counts. No further transformation of the data could remove this effect and therefore the analysis of variance has been applied.

Table 1. *Number of arrested mitoses per mouse in five fields at ten different times after the injection of oestrone*

Mouse	Time (hr.)									
	0	6	12	18	24	30	36	42	48	54
1	0	2	5	51	27	171	128	*	43	1
2	1	2	0	35	166	193	234	22	177	14
3	0	0	2	38	184	182	238	105	99	23
4	2	0	3	21	248	173	182	177	27	37
5	2	1	0	11	*	150	201	103	15	30

\* Missing observations.

Table 2. *Analysis of variance of the data of Table 1 following the transformation  $Y_1 = \log_{10}(Z_1 + 1)$ , where  $Z_1$  is the number of arrested mitoses*

Source of variation	D.F.	Mean square	F	P
Times:	(9)			
Linear	1	11.00	131	< 0.001
Quadratic	1	14.23	169	< 0.001
Cubic	1	2.71	32	< 0.001
Quartic	1	1.64	19	< 0.001
Quintic	1	0.09	1.1	> 0.05
Remainder	4	0.17	2.0	> 0.05
Error	38*	0.084		

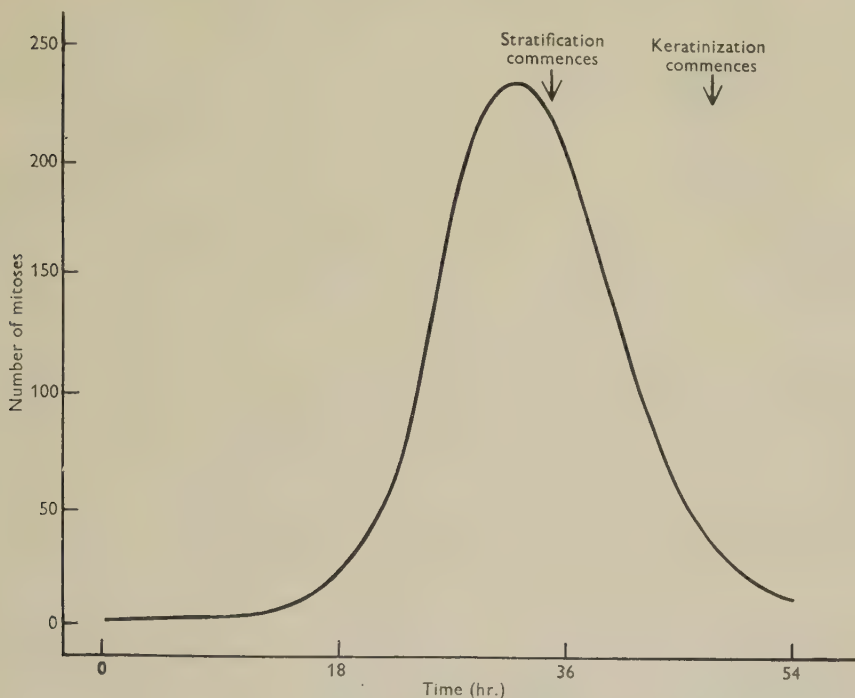
\* In this Table and Table 4 two degrees of freedom have been deducted for missing observations; before computing the analysis of variance, estimates of the missing observations were made (Cochran & Cox, 1950).

The analysis of variance is shown in Table 2. The sum of squares, which may be attributed to time differences, has been partitioned by means of orthogonal polynomial coefficients to the fifth order (Fisher & Yates, 1953). Since the quintic and remainder items are not significant the data can be described by a polynomial in  $X$  of the fourth order, where  $X$  is the time after injection of oestrone. The polynomial has been computed, inversely transformed by  $Z_1 = \text{antilog}_{10} Y_1 - 1$ , and plotted in Text-fig. 1.

The results demonstrate that following a latent period of about 12 hr. after the injection of oestrone, an exponential increase in the number of arrested mitoses takes place. The maximum number of arrested mitoses occurs between 30 and 36 hr., and is followed by an exponential decline. By 54 hr. the number of arrested mitoses is almost back to that seen initially.

(ii) *Cell layers.* The observations are given in Table 3. The variability of these observations had a similar pattern to the variability of the observations on arrested mitoses. After making the transformation  $Y_2 = \log_{10} Z_2$ , where  $Z_2$  is the number of

cell layers, Bartlett's test was applied, using the first approximation. The variances of the data can be considered homogeneous ( $\chi^2_{[9]} = 15.8$ ,  $0.2 > P > 0.1$ ).



Text-fig. 1. The number of arrested mitoses in five fields at various times after the intravaginal injection of oestrone.

Table 3. Average number of cell layers per mouse at five positions at ten different times after the injection of oestrone. The figures in this table are in one-to-one correspondence with those in Table 1

Mouse	Time (hr.)									
	0	6	12	18	24	30	36	42	48	54
1	2.0	2.8	2.6	7.0	3.4	4.2	6.8†	*	8.4‡	6.0†
2	2.4	2.4	2.6	3.6	3.8	4.4	7.6†	6.8†	4.8†	6.0†
3	2.4	1.8	2.2	3.0	7.0	4.4	4.6	4.6	11.8‡	8.6‡
4	2.0	2.0	2.2	2.4	4.6	5.0	6.8†	4.2	8.6‡	9.4‡
5	2.0	2.8	3.2	2.4	*	4.0	7.0†	6.4	6.8‡	5.2†

\* Missing observations.

† Epithelium stratified with flattened upper layers.

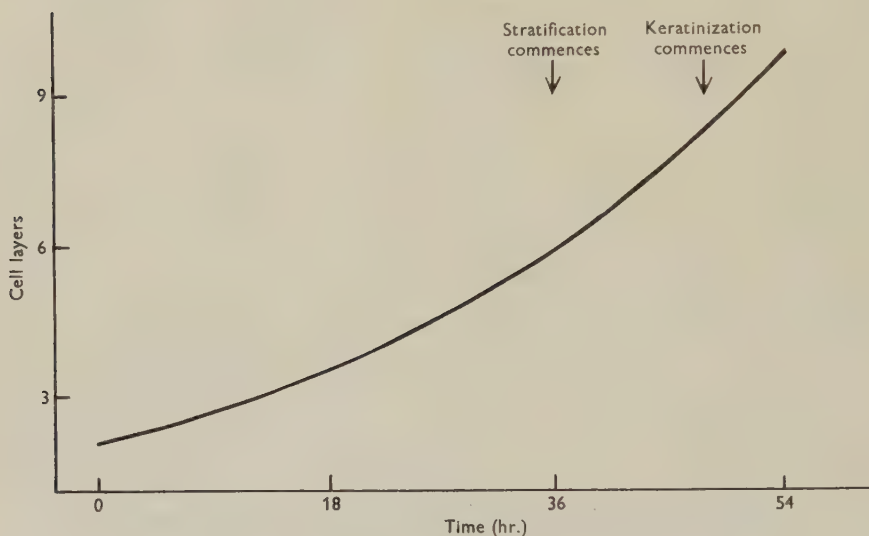
‡ Epithelium keratinized.

The analysis of variance is shown in Table 4, and is of the same form as that in Table 2. The data may be described by an equation linear in  $X$ . Following the inverse transformation  $Z_2 = \text{antilog}_{10} Y_2$  the equation has been plotted (Text-fig. 2). It can be seen that throughout the whole period the number of cell layers increases exponentially.



Table 4. *Analysis of variance of the data of Table 3 following the transformation  $Y_2 = \log_{10} Z_2$ , where  $Z_2$  is the average number of cell layers*

Source of variation	D.F.	Mean square	F	P
Times:	(9)			
Linear	1	1.667	129	< 0.001
Quadratic	1	0.016	1.3	> 0.05
Cubic	1	0.011	< 1	> 0.05
Quartic	1	0.003	< 1	> 0.05
Quintic	1	0.014	1.1	> 0.05
Remainder	4	0.013	1.0	> 0.05
Error	38	0.0129		



Text-fig. 2. The number of cell layers at various times after the intravaginal injection of oestrone.

#### *Qualitative observations*

The resting epithelium is 2-3 cells thick, and only contains occasional mitoses (Pl. 1, fig. 1). This finding shows that colchicine itself does not stimulate mitosis. Once divisions commence, arrested mitoses are seen throughout the 3-4 cell layers of the epithelium (Pl. 1, fig. 2). When the number of arrested mitoses is at a maximum at 36 hr., many mitoses are seen in both the stratum germinativum and stratum spinosum (Pl. 1, fig. 3). At this stage stratification (i.e. the attainment of a stratified squamous epithelium) is seen in some animals (see Table 3). After this period the number of mitoses falls and those that are seen are in the basal layer of the epithelium (Pl. 1, fig. 4). Keratinization begins beneath a superficial layer of cells about 48 hr. after the injection of oestrone. Stratification and keratinization appear to be unaffected by the administration of colchicine and these changes are identical with those described by Biggers (1953*b*).

Hanson (1947), in studies of the epidermis of the mouse, states that occasional mitoses may be seen in the stratum spinosum for a short period after its formation. In the case of the vagina a spinosum layer rapidly develops under the action of oestrone and dividing cells occur throughout this layer.

## DISCUSSION

Biggers & Claringbold (1954) have studied the conditions which lead to a maximum percentage response of mice to intravaginally administered oestrogens. One variable studied was the period over which the oestrogens should be administered. The maximum response was obtained with a time interval of 36 hr., i.e. the last injection was made 36 hr. after the first. Previously Marrian & Parkes (1929), in studies where the oestrogens were administered subcutaneously, found a similar result. It is important that this period coincides with the time of increased mitotic rate. Biggers & Claringbold (1954) suggested that a period of 36–48 hr. is required by the epithelium for the most efficient utilization of the injected oestrogen. The present work suggests that the oestrogens are required over this period to produce the increased mitotic rate.

Jeener (1948) concluded that at 24 hr. after the subcutaneous injection of oestradiol-3:17 $\beta$  in ovariectomized mice there were large increases in the desoxyribonucleic acid (D.N.A.), ribonucleic acid (R.N.A.), and alkaline phosphatase. The work of Gothié (1952), who studied the distribution of  $^{32}\text{P}$  in the vagina of the ovariectomized mouse after the subcutaneous injection of oestradiol-3:17 $\beta$  benzoate supports the findings of Jeener. The increase of D.N.A. indicates that synthesis of nuclear material is taking place, a finding which is consistent with the present work. The increases in R.N.A. and alkaline phosphatase are indicative of general protein synthesis and growth, also demonstrated in our work.

Histochemical studies (Biggers, 1953*b*) and biochemical studies (Balmain, Biggers & Claringbold, 1955) have shown that glycogen deposition in the vagina is associated with keratinization. Thus the appearance of glycogen in the vaginal epithelium is not associated with the period of increased mitotic activity. Bullough (1950) has suggested that oestrone may stimulate mitosis by producing hyperglycaemia. This possibility has been excluded in the present work by the use of the intravaginal technique. It seems that changes in carbohydrate metabolism are most likely to occur during the period of high mitotic activity and protein synthesis, and that the accumulation of glycogen which occurs after the start of keratinization, is an effect of secondary importance.

Following the administration of oestrogen the vaginal epithelium responds rapidly with large increases in mitotic rate. It seems that this organ and method of study would be of value in further studies of mitosis.

## SUMMARY

1. The mitotic activity and number of cell layers has been studied in the vaginal epithelium of the ovariectomized mouse following the intravaginal administration of oestrone.

2. After a latent period of about 12 hr. the mitotic rate increases exponentially until 30–36 hr. after injection. It then declines exponentially and is almost back to the initial level by 54 hr.

3. The number of cell layers increases exponentially throughout the period of 54 hr. studied, stratification commencing at 36 hr. and keratinization at 48 hr.

4. It is suggested that the oestrogen must be present throughout the period of increased mitotic activity. The period of increased glycogen content of the epithelium does not coincide with the period of increased mitotic activity.

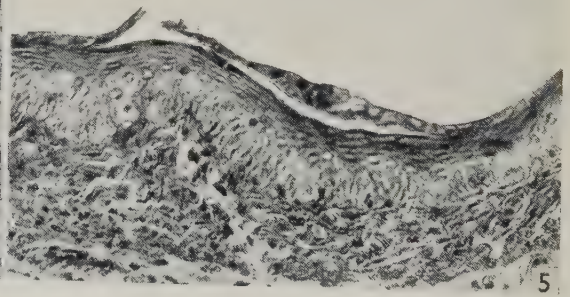
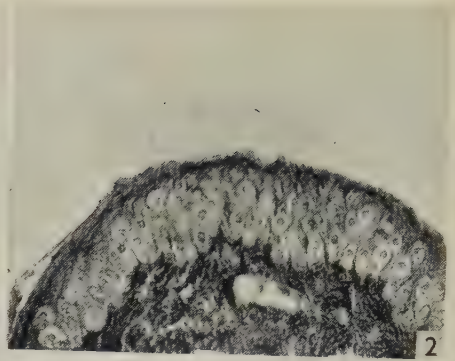
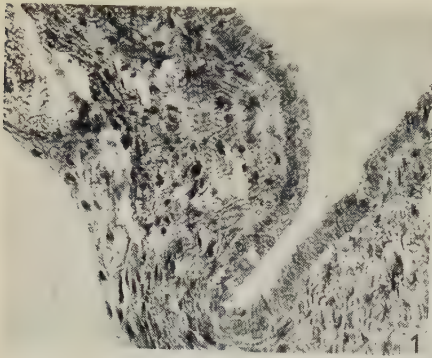
This work was assisted by grants from the Wool Industry Fund and the Commonwealth Bank of Australia.

#### REFERENCES

- ALLEN, E., SMITH, G. M. & GARDNER, W. U. (1937). Accentuation of the growth effect of theelin on genital tissues of the ovariectomized mouse by arrest of mitosis with colchicine. *Amer. J. Anat.* **61**, 321-341.
- BALMAIN, J. H., BIGGERS, J. D. & CLARINGBOLD, P. J. (1955). Glycogen, wet weight and dry weight changes in the vagina of the mouse. In the press.
- BIGGERS, J. D. (1951). Observations on the intravaginal assay of natural oestrogens using aqueous egg albumin as the vehicle of administration. *J. Endocrin.* **7**, 163-171.
- BIGGERS, J. D. (1953*a*). The effect of different protein solvents on the dose-response line for the intravaginal administration of oestrone in mice. *J. Endocrin.* **9**, 136-144.
- BIGGERS, J. D. (1953*b*). The carbohydrate components of the vagina of the normal and ovariectomized mouse during oestrogenic stimulation. *J. Anat., Lond.*, **87**, 327-336.
- BIGGERS, J. D. & CLARINGBOLD, P. J. (1954). Optimum conditions for the local (intravaginal) action of oestrogens. *Aust. J. Biol. Sci.* **7**, 118-139.
- BRUES, A. K. (1951). Discussion of paper by Levine (1951).
- BULLOUGH, W. S. (1942). Effect of oestrin injections on the mouse ovary. *Nature, Lond.*, **149**, 271-272.
- BULLOUGH, W. S. (1950). The mitogenic actions of starch and oestrone on the epidermis of the adult mouse. *J. Endocrin.* **6**, 350-361.
- BULLOUGH, W. S. (1952). The energy relations of mitotic activity. *Biol. Rev.* **27**, 133-168.
- COCHRAN, W. G. (1947). Some consequences when the assumptions for the analysis of variance are not satisfied. *Biometrics*, **3**, 22-38.
- COCHRAN, N. G. & COX, G. M. (1950). *Experimental Designs*. New York: Wiley.
- COUJARD, R. (1943). Le rôle du sympathique dans les actions hormonales. *Bull. biol.* **77**, 120-223.
- EMMENS, C. W. (1942). The differentiation of oestrogens from pro-oestrogens by the use of spayed mice possessing two separate vaginal sacs. *J. Endocrin.* **3**, 174-177.
- FISHER, R. A. & YATES, F. (1953). *Statistical Tables for Biological, Agricultural and Medical Research*. 4th ed. Edinburgh: Oliver and Boyd.
- GOTHIÉ, S. (1952). Méthode histochemique d'étude de la répartition du P<sup>32</sup> au cours de la croissance provoquée. *Arch. Biol.* **63**, 441-453.
- VON HAAM, E. & CAPPEL, L. (1940). Effect of hormones upon cells grown *in vitro*. I. The effect of sex hormones upon fibroblasts. *Amer. J. Cancer*, **39**, 350-353.
- HANSON, J. (1947). The histogenesis of the epidermis in the rat and mouse. *J. Anat., Lond.*, **81**, 174-197.
- HARDY, M. H., BIGGERS, J. D. & CLARINGBOLD, P. J. (1953). Vaginal cornification of the mouse produced by oestrogens *in vitro*. *Nature, Lond.*, **172**, 1196.
- JEENER, R. (1948). Acides nucléiques et phosphatases au cours de phénomènes de croissance provoqués par l'oestradiol et la prolactine. *Biochim. biophys. Acta*, **2**, 439-453.
- KAHN, R. H. (1954). Effect of oestrogen and of vitamin A on vaginal cornification in tissue culture. *Nature, Lond.* **174**, 317.
- LEVINE, M. (1951). The action of colchicine on cell division in human cancer, animal, and plant tissues. *Ann. N.Y. Acad. Sci.* **51**, 1365-1408.
- MARRIAN, G. F. & PARKES, A. S. (1929). The assay of oestrin. *J. Physiol.* **67**, 389-401.
- RAO, C. R. (1952). *Advanced Statistical Methods in Biometric Research*. New York: Wiley.
- ROBSON, J. M. & ADLER, J. (1940). Site of action of oestrogens. *Nature, Lond.*, **146**, 60.
- STEIN, K. F. & ALLEN, E. (1942). Attempts to stimulate proliferation of the germinal epithelium of the ovary. *Anat. Rec.* **82**, 1-9.







BIGGERS AND CLARINGBOLD—MITOTIC ACTIVITY IN THE VAGINA

EXPLANATION OF PLATE

All sections have been stained with haemotoxylin and eosin.  $\times 360$ .

- Fig. 1. Vaginal wall of an ovariectomized mouse 9 hr. after the injection of 0.1 mg. colchicine. No arrested mitoses are seen.
- Fig. 2. Vaginal wall of an ovariectomized mouse 18 hr. after the injection of  $6.4 \times 10^{-3}$   $\mu$ g. oestrone, and 9 hr. after the injection of 0.1 mg. colchicine. Many arrested mitoses are seen in the three to four layers of epithelial cells.
- Fig. 3. Vaginal wall of an ovariectomized mouse 36 hr. after the injection of  $6.4 \times 10^{-3}$   $\mu$ g. oestrone and 9 hr. after the injection of 0.1 mg. colchicine. Many arrested mitoses are seen chiefly in the basal layers of the epithelium.
- Fig. 4. Vaginal wall of an ovariectomized mouse 48 hr. after the injection of  $6.4 \times 10^{-3}$   $\mu$ g. oestrone, and 9 hr. after the injection of 0.1 mg. colchicine. Only a few arrested mitoses are seen in the epithelium which has begun to keratinize.
- Fig. 5. Vaginal wall of an ovariectomized mouse 54 hr. after the injection of  $6.4 \times 10^{-3}$   $\mu$ g. oestrone, and 9 hr. after the injection of 0.1 mg. colchicine. Only two or three arrested mitoses are seen in the epithelium.



## REVIEW

*Die Entwicklung und Morphologie des Chondrokraniums von Myotis* Kaup. By HANS FRICK. Georg Thieme Verlag, Stuttgart, 1954. Kartoniert D.M. 14.40.

This excellently produced monograph on the development of the skull of *Myotis* is the third contribution from Senckenberg Institute in Frankfurt on the ontogeny of the cranium in Cheiroptera. In itself and when considered in conjunction with the papers by Sitt on the primordial and osteocranium of *Rhinolophus* and of Starek on the chondrocranium of *Pteropus*, the present contribution goes a long way to fill that gap in our knowledge of the development of the mammalian skull which the paucity of information on bats provided, and which was deplored by de Beer in his classical volume.

Dr Frick provides a well illustrated account of a number of stages in skull differentiation in *Myotis myotis* and of two stages in *Myotis capucinii*. A detailed morphological description, such as is given, does not readily permit of general review or criticism. The author himself provides four pages of summary of his essential conclusions containing no fewer than sixty-three points! It can, however, be written that the descriptions are clear, the deductions reasonable, and the general presentation agreeable. There is a good bibliography.

J. D. BOYD

## BOOKS RECEIVED

*Anatomy. Regional and Applied.* By R. J. LAST. London: J. and A. Churchill Ltd. 1954. 55s.

*Basic Anatomy.* By G. A. G. MITCHELL and E. L. PATTERSON. Edinburgh and London: E. and S. Livingstone Ltd., 1954. 45s.

*Gray's Anatomy. Descriptive and Applied.* By T. B. JOHNSTONE and J. WHILLIS. 31st Edition. London: Longmans, Green and Co., 1954. 100s.

*Anatomie humaine. Descriptive et topographique.* Tome I. *Tête et cou.* Tome II. *Tronc.* Tome III. *Membres. Système nerveux central.* By H. ROUVIÈRE. Septième édition révisée par G. Cordier. Paris: Masson et Cie, 1954. Cartonné 15,000 Fr.

# THE PLACODAL RELATIONS OF THE NEURAL CREST IN THE DOMESTIC CAT

By GWEN HALLEY

*Department of Veterinary Anatomy, University of Bristol and  
Division of Histology, Royal Veterinary College, London*

## INTRODUCTION

In an attempt to trace the establishment of the sensory ganglia of the cranial nerves of the cat there are two main aspects to be considered. They are, first, the formation and fate of the cranial neural crest and its participation in the formation of the ganglia; and, second, the contribution to the ganglia of cells from placodal thickenings of the general ectodermal surface of the head.

From the extensive work of Landacre (1908, 1910*a, b*, 1912, 1921, 1927, 1931, 1932, 1933) and others including Reed (1916), Stone (1922, 1924, 1928*a-c*, 1929*a, b*), Kostir (1924) and Knouff (1927, 1935) there has emerged the concept of a general pattern of development of the functional components of the ganglia of the dorsal series of cranial nerves of the vertebrates. The conclusion reached is that the sensory ganglia of the vertebrate head have a dual origin, coming in part from the cranial neural crest and in part from placodal thickenings of the dorso-lateral and epibranchial head ectoderm. Furthermore the general visceral and general somatic components arise from the neural crest, while the acoustic and lateral line ganglionic components (special somatic) come from the dorso-lateral placodes, and the gustatory fibres (special visceral) from cells derived from the epibranchial placodes. These views are summarized by Kappers (1941). The neural crest, apart from forming ganglion cells, contributes to the general head mesenchyme including the cartilage of the branchial arches.

These conclusions have been formulated from observations and experiments upon fishes and amphibia, where it has been possible to distinguish the neural crest cells histologically up to late stages of differentiation (Landacre, 1912, 1921), and where the established ganglia can be seen to be composed of distinct masses of a particular type of component traceable to specific sites of origin (Landacre, 1921; Knouff, 1927). Experimental work in the chick (Yntema, 1944) indicates that the cranial ganglia in the bird are also arising in part from the neural crest and in part from dorso-lateral and epibranchial placodes.

In mammals the conditions are more specialized. Also the histological picture can be interpreted with less certainty, for the mammalian neural crest cells and placodal cells are not strikingly different from those of the general head mesenchyme; and they tend to differ from those of fishes and amphibia in that they migrate within the head in diffuse streams, rather than in compact masses, to their destinations. These facts, together with the greater difficulty of obtaining material and the lack of experimental evidence have led to much controversy regarding the role of the placodes in the mammals (Adelmann, 1925; Bartelmez, 1924; Bartelmez & Evans,

1926; Baxter & Boyd, 1939; Campenhout, 1935*a, b*, 1936, 1937*a*, 1948; Coërs, 1946; Da Costa, 1921; Frazer, 1925; Holmdahl, 1928; Landacre, 1932).

However, there is considerable histological evidence in embryos of the domestic cat to suggest that the same general pattern of development is being followed in this mammal as is taking place in the fishes and amphibia. Furthermore the concept of the epibranchial placodes as ganglion-forming structures offers an explanation of the morphological changes taking place in the region of the cervical sinus (Frazer, 1925).

#### MATERIALS AND METHODS

This account is based upon the study of a series of 54 cat embryos ranging from the 2-somite stage to the size of 15 mm. crown-rump length, covering the period from the 13th to the 22nd day of pregnancy.

The fixatives employed were Zenker-formol and Bouin's fluid; and the embryos were cleared in toluol and embedded in paraffin wax. The sections were stained in Ehrlich's haematoxylin and eosin.

The young embryos were cut transversely or longitudinally, while those of 5 mm. or more in length were cut transversely, coronally or sagittally. The coronal plane proved the most satisfactory for the examination of the cranial ganglia in relation to their placodes.

The ages of the specimens prepared by the writer were estimated from a table of foetal measurements at known stages after mating, which was kindly lent by Professor E. C. Amoroso, who also provided some of the completely prepared series. For description of the brain of the young embryos the terminology of Adelman (1925) has been adopted.

#### OBSERVATIONS

##### *5-7-Somite stage, 13-14 days*

At the 5-somite stage, while the neural plate is still almost flat, the trigeminal neural crest (rostral neural crest of Adelman, 1925) begins to form in the region just anterior to the pre-otic sulcus—that is at the junction of the future mid- and hind-brain territories (Text-fig. 1).

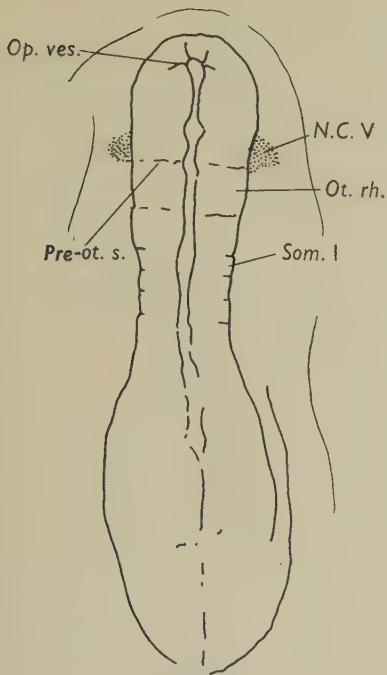
In 7-somite cat embryos the rostral flexure of the neural plate has carried the future fore-brain ventrally and the trigeminal neural crest now lies at the topographical anterior end of the embryo (Text-figs. 2 and 3). The anterior cephalic mesenchyme is scanty and appears to be arising from the distal end of the trigeminal neural crest; this confirms the observation of Holmdahl (1928) in the same species.

##### *8-14-Somite stage, 14-15 days*

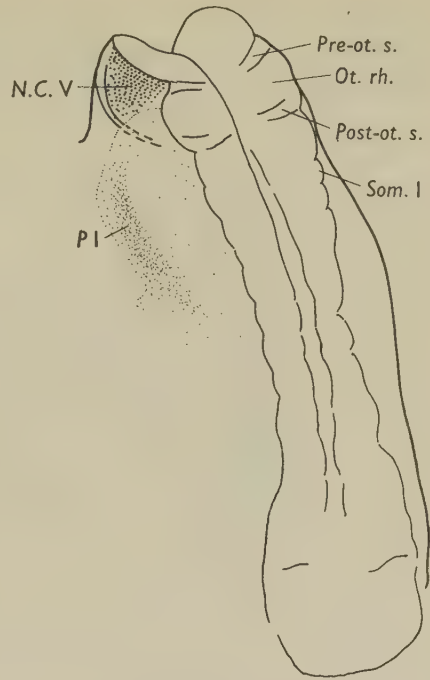
During this time the neural plate rolls up to form a tube while the rostral flexure persists.

*Trigeminal neural crest.* The cells of the anterior part of the trigeminal neural crest become diffusely scattered, but the most posterior part of this crest remains compact and is the anlage of the trigeminal ganglion (Text-figs. 4-6; Pl. 1, fig. 15). Where the scattered cells of the anterior part of the trigeminal neural crest reach the contact of the optic vesicles with the overlying ectoderm, at the 14-somite stage, there is slight proliferative activity of the somatic ectoderm.





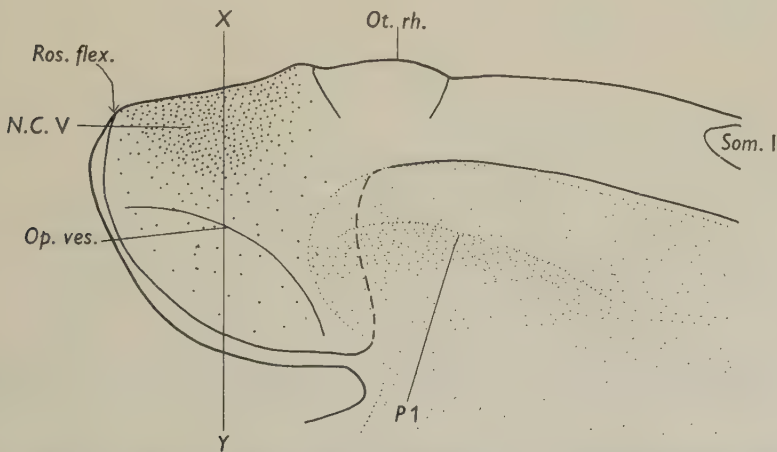
Text-fig. 1.



Text-fig. 2.

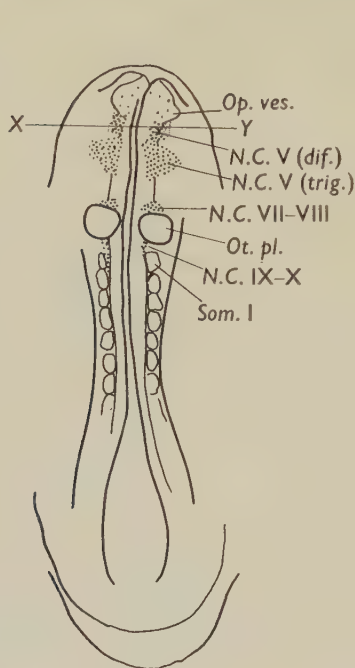
Text-fig. 1. Diagram of dorsal view of a 5-somite cat embryo based upon a wax model of the specimen.

Text-fig. 2. Diagram of dorso-lateral view of 7-somite cat embryo, based upon a photograph and wax model of the specimen.



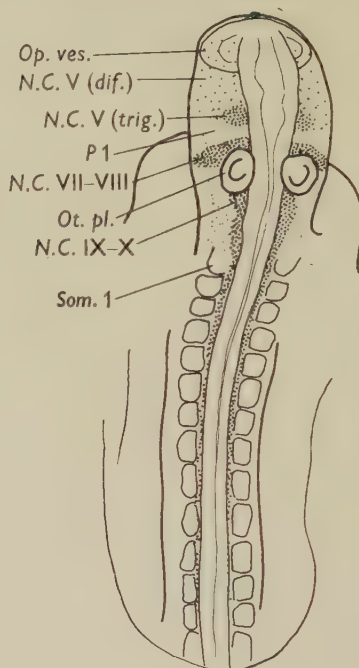
Text-fig. 3. Diagram of lateral view of model of anterior end of the same cat embryo as shown in Text-fig. 2. The lateral somatic ectoderm has been dissected away to expose the trigeminal neural crest, optic vesicle and pharynx. A photomicrograph of section X-Y is shown on Pl. 1, fig. 14. View from left side.

*Acoustico-facial neural crest.* The acoustico-facial neural crest appears at the anterior margin of the otic placode at the 8-somite stage. In 14-somite cat embryos it is arising from a considerable dorso-ventral extent of the neural tube and from the



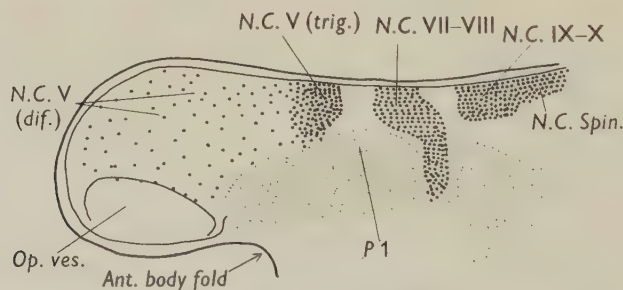
Text-fig. 4.

Text-fig. 4. Diagram of dorsal view of 8-somite cat embryo based on photograph and wax model. A photomicrograph of a section at X-Y is shown on Pl. 1, fig. 15.



Text-fig. 5.

Text-fig. 5. Diagram of dorsal view of 14-somite cat embryo based on photograph and wax model.



Text-fig. 6. Diagram of lateral view of cranial end of 14-somite cat embryo. The lateral somatic ectoderm has been dissected away. View from left side.

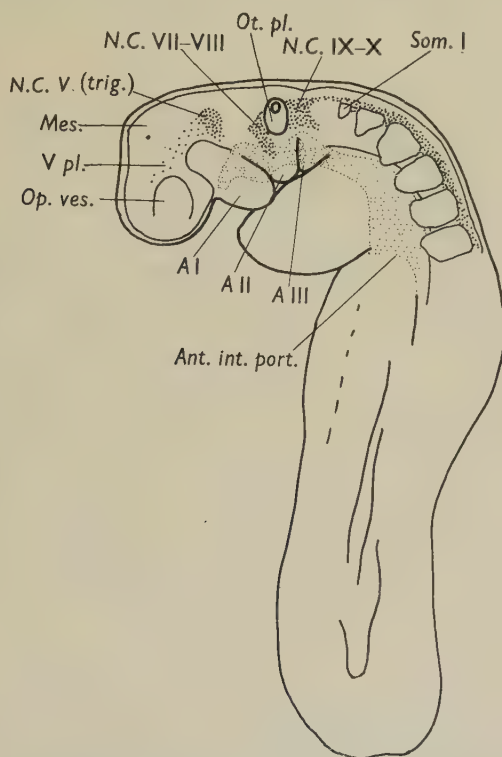
juxta-neural somatic ectoderm, from whence it extends ventro-laterally and merges with the dense tissues in the hyoid arch (Text-fig. 6).

From the neuro-somatic junction just caudal to the otic placode the vago-glossopharyngeal crest arises (Text-figs. 4, 5).

*20-Somite stage, 15 days*

The neural tube has developed mesencephalic and cervical flexures, and the pharyngeal clefts are appearing on the lateral surfaces of the head (Text-fig. 7).

*Trigeminal neural crest.* The trigeminal neural crest is no longer in process of arising from the neuro-somatic junction. Its anterior part has become quite unrecognizable, but its posterior portion forms the anlage of the trigeminal ganglion.



Text-fig. 7. Diagram of 20-somite cat embryo, based on photograph taken from the ventral side after torsion has taken place, and on a wax model of the cranial end of the specimen.

Overlying the ganglion and extending anteriorly from it the ectoderm has become diffusely proliferative. This ectoderm corresponds with the ectoderm of 'area V' of Coërs (1946) and is the same epidermis beneath which, at the 14-somite stage, the scattered cells of the anterior part of the trigeminal neural crest were seen.

*Acoustico-facial neural crest.* The acoustico-facial crest extends as a solid column of epithelioid cells—exhibiting frequent mitoses but no sign of cellular degeneration—from the wall of the hind brain down into the hyoid arch. No boundary can be distinguished between the neural crest and the dense branchial mesoderm.

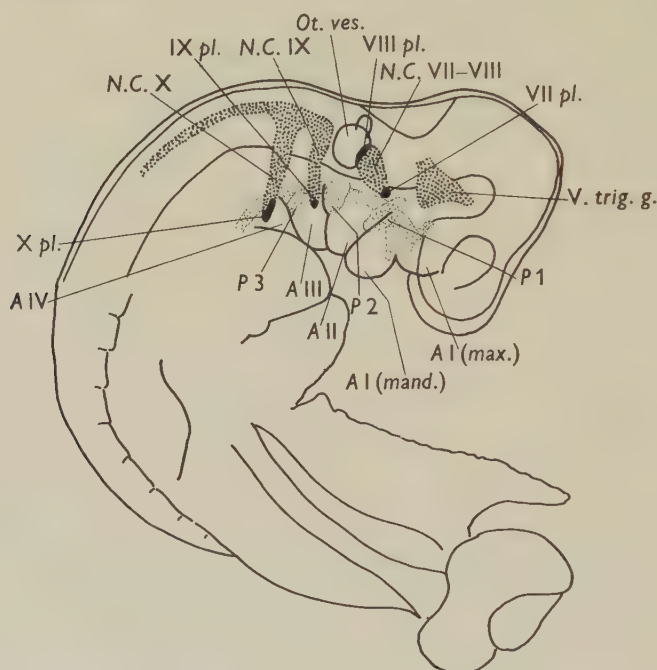
*Vago-glossopharyngeal neural crest.* Many of the cells of the distal part of this crest seem to be separating off and passing into the mesoderm of the third and fourth branchial arches.



5 mm. (C.R. length) stage, 16-17 days

Four pharyngeal arches are clearly distinguishable on the surface of the head (Text-fig. 8). The third and fourth arches lie in a sunken triangular area which is the cervical sinus.

*Trigeminal neural crest.* The anlage of the trigeminal ganglion lies against the alar lamina of the rhombencephalon. The dorsal surface of the ganglion is separated from the lateral somatic ectoderm by a considerable thickness of loose mesenchyme, but its distal end passes out ventrolaterally towards the ectoderm of the mandibular



Text-fig. 8. Diagram of lateral view of 5 mm. (C.R. length) cat embryo based upon a photograph and wax model.

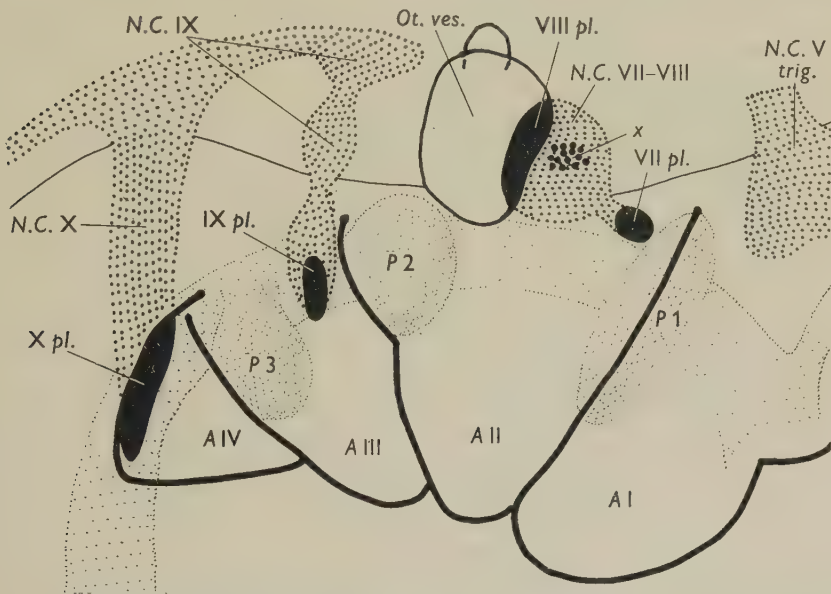
arch (Pl. 1, fig. 16). The cells in this distal region of the crest are more loosely packed and occupy the zone between the epithelioid proximal crest and the condensed branchial mesoderm. Numerous pycnotic nuclear fragments are present within the neural crest and the dorsal parts of the branchial arch. The ectoderm overlying the trigeminal ganglion, and extending anteriorly as far as the optic vesicles, shows punctate proliferative activity. Nodules and strands of epithelial cells may be seen in all stages of detachment from the undersurface of the epidermis. These cells appear either to enter the ganglion or to take up a position lateral to the anterior cardinal vein at the site of the future ophthalmic ramus V.

*Acoustico-facial neural crest.* The acoustico-facial neural crest consists of a large mass of elongated epithelioid cells extending ventrolaterally from the wall of the rhombencephalon. It then tapers abruptly before passing out towards the epi-branchial ectoderm just caudal to the hyomandibular cleft (Pl. 2, fig. 19). Where the

acoustico-facial crest rests against the alar wall of the rhombencephalon cells are leaving the neural tube and passing into the crest ('X' in Text-fig. 9).

A diffuse mass of cells projects from the epibranchial ectoderm of the hyoid arch towards the distal end of the acoustico-facial crest (Pl. 2, fig. 19). Between the neural crest and the placodal mass cellular degeneration is taking place, as evidenced by the presence of numerous pycnotic nuclear fragments.

*Vago-glossopharyngeal crest.* The vago-glossopharyngeal crest has differentiated into separate vagal and glossopharyngeal portions. The *glossopharyngeal neural crest* has differentiated into a dorsal and a ventral, or petrosal, mass. From the epibranchial ectoderm of the third pharyngeal arch a large epithelial mass extends



Text-fig. 9. Diagram of the relations of the neural crest to the pharyngeal pouches and arches in the 5 mm. (c.r. length) cat embryo. At 'X' cells are leaving the brain wall and are entering the acoustico-facial neural crest. View from right side of embryo.

into the underlying mesenchyme towards the ventral portion of the glossopharyngeal neural crest (Pl. 2, fig. 22). The *vagal neural crest* consists of loosely aggregated cells extending towards the dorsal end of the fourth arch, from the ectoderm of which placodal migration is taking place (Text-figs. 8, 9).

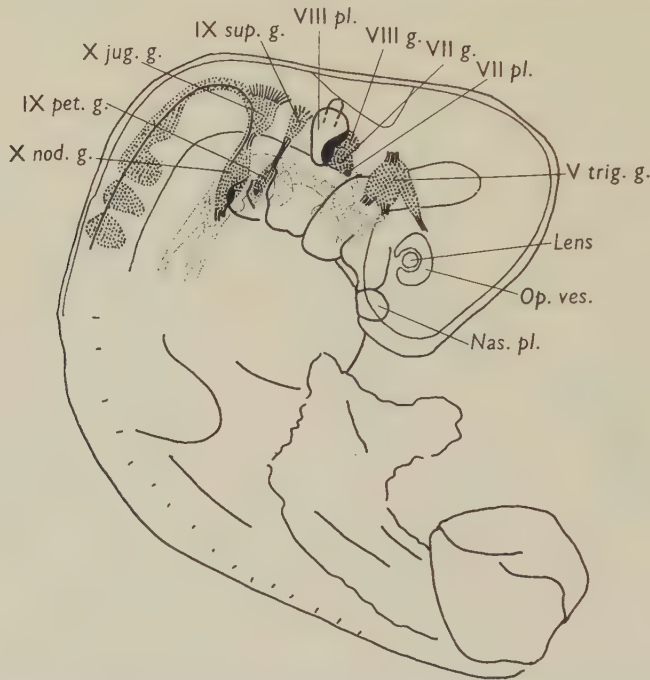
#### *7-8 mm. (c.r. length) stage, 18-19 days*

The third and fourth pharyngeal arches are relatively smaller than at the 5 mm. stage. The cervical sinus has been deepened by the growth of the surrounding tissue, and the anterior part of the third arch and posterior part of the fourth arch are thus overlapped. All four pharyngeal pouches are in contact with corresponding ectodermal clefts (Text-fig. 10).

*Trigeminal ganglion.* The trigeminal ganglion is a large fibro-cellular mass; it is separated from the lateral somatic ectoderm by a thin layer of mesenchyme, across

which occasional cellular strands and nodules are passing from the epidermis to the ganglion. The ophthalmic lobe is prolonged forwards lateral to the anterior cardinal vein as a predominantly cellular ophthalmic ramus. The ectoderm overlying the anterior cardinal vein still shows irregular thickenings, and nodules and strands of epithelial cells are leaving the epidermis and passing towards the ophthalmic lobe and ramus (Pl. 1, figs. 17, 18).

*Acoustic and facial ganglia.* The *facial (geniculate) ganglion* is now seen in the angle between the roof of the hyomandibular pouch and the epibranchial ectoderm. Its



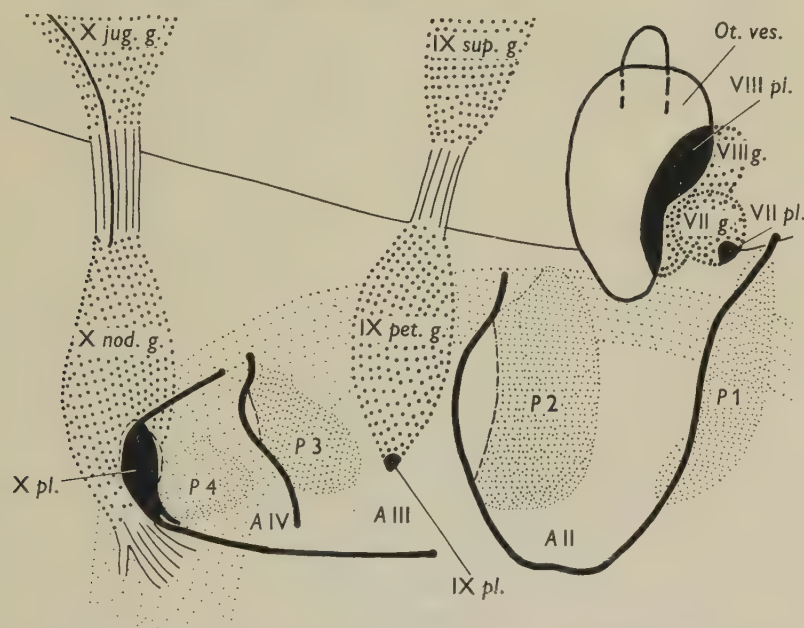
Text-fig. 10. Diagram of lateral view of 7 mm. (c.r. length) cat embryo, based upon a photograph and graphic reconstruction.

distal end is continuous with the epibranchial placode of the hyoid arch (Pl. 2, fig. 20). The cells in the placode are small, rounded and closely packed; passing towards the body of the geniculate ganglion a gradual transition into neuroblasts is apparent. Dorsally the geniculate ganglion is continuous with the acoustic primordium. The *acoustic ganglion* rests against the antero-ventral wall of the otic vesicle. The greater part of the wall of the otocyst is clearly defined from the surrounding tissues; but where it is in contact with the acoustic primordium the basement membrane is indistinct and streams of cells can be seen migrating from its wall into the ganglion primordium (Pl. 2, fig. 21).

*Vagal and glossopharyngeal ganglia.* The superior ganglion of nerve IX consists of a group of neural crest cells clustered around the nerve root at its junction with the brain wall. The body of the petrosal ganglion lies immediately behind the second pharyngeal pouch, and its distal end is fused with the epibranchial ectoderm of the



third pharyngeal arch. Two zones can be distinguished within the petrosal ganglion; the proximal part consists of elongated neuroblasts, while the distal part is composed of irregular epithelial cells with more lightly-staining cytoplasm. The epidermis is continuous with the latter portion. The petrosal neural crest and the placodal ingrowth present at the 5 mm. stage have fused to form the proximal and distal portions of the ganglion (Pl. 2, fig. 23). At the origin of the vagus nerve from the brain wall, numerous small irregular neural crest cells are aggregated to form the jugular ganglion. From this ganglion a long fibro-cellular root extends down to the



Text-fig. 11. Diagram of the relations of the acoustico-facial and vago-glossopharyngeal neural crests to the pharyngeal arches and pouches in a 7 mm. (C.R. length) cat embryo. The closing plate of pouch 1 is visible on the surface, but those of pouches 2, 3 and 4 are all partially hidden by overgrowth of the surrounding tissues, in which regions they are indicated by broken lines. The broken line surrounding the placode of the vagus indicates the margin of a shallow pit. View from right side.

nodose ganglion. The nodose ganglion lies immediately behind the closing plate of the fourth arch; it is intimately fused with the epibranchial ectoderm of the fourth arch, and in this region cells can be seen passing out of the epibranchial placode into the ganglion primordium. The dorso-caudal end of arch four—its placodal portion—is becoming submerged by the growth of the tissues around the caudal border of the cervical sinus (Text-fig. 11). The nodose ganglion consists of a proximal portion composed of elongated neuroblasts, and a distal portion composed of irregular epithelial cells continuous with the epibranchial ectoderm of arch IV.

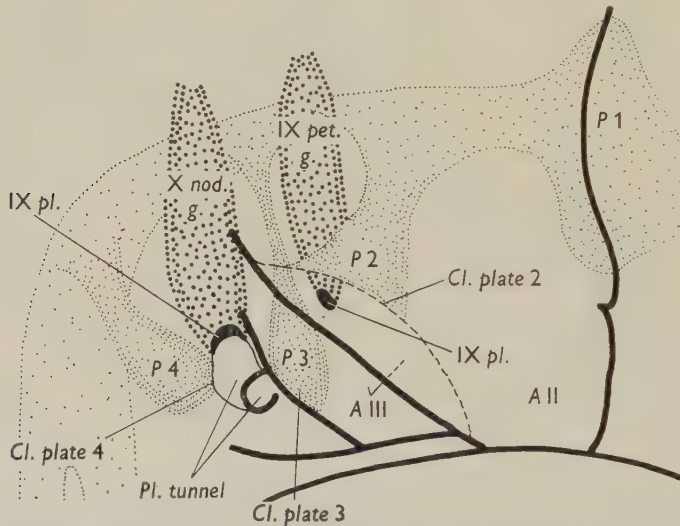
#### *9 mm. (C.R. length) stage, 20 days*

Of the general morphological changes that distinguish this from the last described stage, those in the region of the caudal pharyngeal arches are the most significant in

the consideration of the cranial ganglia (Text-fig. 12). The hyoid arch is large, and its caudal border overhangs the closing plate of the second pouch and the anterior part of arch III—including the portion bearing the placode associated with the petrosal ganglion. The closing plate of the third pouch remains visible on the surface. On the fourth arch a small pit can be seen, which leads down to the placode of the nodose ganglion; the closing plate of pouch 4 also lies within this pit.

*Trigeminal ganglion.* The proliferative activity of the epidermis overlying the trigeminal ganglion has waned, but both the ophthalmic and maxillo-mandibular portions are still receiving occasional contributions from the ectoderm.

*Facial (geniculate) ganglion.* The facial ganglion is now composed of rounded neuroblasts with large lightly-staining nuclei and acidophilic cytoplasm. The ganglion is no longer in contact with the epibranchial ectoderm.



Text-fig. 12. Diagram of the relations of the petrosal ganglion of nerve IX and the nodose ganglion of nerve X to the pharyngeal arches and pouches in a 9 mm. (c.r. length) cat embryo. View from right side.

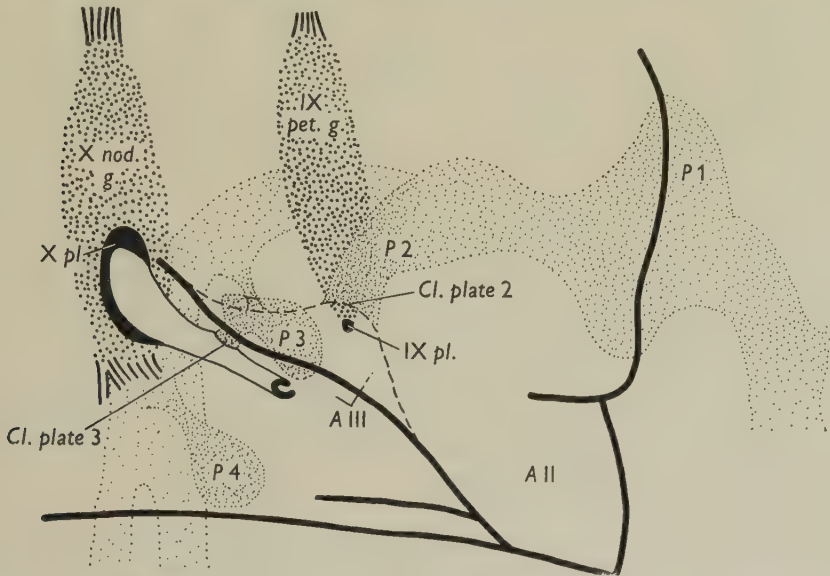
*Acoustic ganglion.* The acoustic ganglion is differentiating into cochlear and vestibular portions. Where the vestibular portion is in contact with the otic vesicle small darkly-staining cells are streaming out of the epithelium into the ganglion.

*Glossopharyngeal ganglia.* The distal end of the petrosal ganglion is still in contact with the epibranchial ectoderm of arch III, but the placodal area is hidden from the surface by the overhanging hinder end of the hyoid arch (Text-fig. 12). The cells of the ganglion which are in contact with the placode are small and irregularly arranged, while those further dorsally are typical elongated neuroblasts.

*Vagal ganglia.* The nodose ganglion is still in intimate contact with its placode, which now lies at the bottom of a pit (Text-fig. 12). Numerous small cells are leaving this placode and are passing into the distal end of the nodose ganglion (Pl. 2, fig. 24). Proximally the ganglion consists of typical neuroblasts.

10 mm. (c.r. length) stage, 21 days

In 10 mm. embryos the growth of the caudal end of the hyoid arch has continued until it overlaps almost all of arch III. The closing plate of the second pouch has been compressed into a solid strand of cells, while the ectoderm of the overhanging portion of the hyoid arch has fused with that of the underlying third arch. The ectodermal pit leading down to the placode of the vagus has elongated and opens on to the surface by a minute pore (Text-fig. 13). The third pharyngeal pouch has thickened and become almost solid, but remains in contact with the ectoderm. Elongation of the pit associated with the vagus placode has incorporated the closing plate of the third pouch into the anterior wall of the tunnel. The fourth pouch, now almost solid, has lost contact with the ectoderm of the tunnel.



Text-fig. 13. Diagram of the relations of the petrosal ganglion of nerve IX and the nodose ganglion of nerve X to the pharyngeal arches and pouches in a 10 mm. (c.r. length) cat embryo. View from right side.

*Trigeminal ganglion.* Placodal activity in relation to this ganglion ceases at this stage.

*Glossopharyngeal ganglia.* The petrosal ganglion loses contact with the epibranchial ectoderm of arch III at this stage. In embryos where a placodal contact remains, the ganglion has a few small cells at its distal tip.

*Vagal ganglia.* The nodose ganglion in the 10 mm. embryo is similar in structure to that in the 9 mm. embryo, but its epibranchial placode now lies at the bottom of a long, tubular pit, and is in fact the proliferative blind end of the tunnel already described.

A few embryos larger than 10 mm. (c.r. length) were examined in order to trace the fate of the placodal tunnel of nerve X. The findings are incorporated in the discussion of the vagal crest.



## DISCUSSION

A. *Trigeminal neural crest*(a) *Formation of trigeminal neural crest*

As early as the 2-somite stage the cephalic end of the neural plate of the cat shows indications of a subdivision into the future brain territories: an otic rhombomere and pre-otic sulcus can be distinguished. However, it is not until the 5-somite stage that any neural crest material can be identified at the margin of the medullary plate (Text-fig. 1).

The trigeminal, or rostral, neural crest is the first to arise in the cat. This is in agreement with the findings of Adelman (1925) in the rat. This mass of neural crest arises from the borders of the future mid- and hind-brain territories and extends caudally as far as the pre-otic sulcus. There is no evidence of the formation of a prosencephalic neural crest in the cat, as has been reported by Bartelmez & Evans (1926) and Baxter & Boyd (1939) in the human, and Campenhout (1937*b*) in the chick. As the neural plate has not yet developed a rostral flexure in 5-somite cat embryos the rostral neural crest does not extend to the topographical anterior end of the embryo (Text-fig. 1). The rostral flexure appears in cat embryos between the 5- and 7-somite stages, and this change in the configuration of the neural plate completely alters the appearance of transverse sections through the rostral neural crest (Pl. 1, fig. 14). As a result of this change the neural crest now lies at the topographical anterior tip of the embryo (Text-figs. 2, 3). Unless the presence of this rostral flexure is taken into account, such sections as shown in Pl. 1, fig. 14, could be interpreted as evidence of the derivation of a prosencephalic neural crest arising from the borders of the optic vesicles. This may explain the claim by Da Costa (1921, 1931) for the presence of a 'peri-optic' neural crest in the guinea-pig. In cat embryos of 7-somites, mesectoderm appears to be arising from the distal end of the trigeminal neural crest (Pl. 1, fig. 14).

(b) *Fate of the anterior (mesencephalic) portion of the trigeminal neural crest*

At the 7-somite stage the trigeminal neural crest extends to the anterior tip of the embryo (Text-figs. 2, 3). In 8-16-somite embryos there appears, on casual inspection, to be a considerable length of the neural tube anterior to the trigeminal anlage which is devoid of neural crest. However, careful examination of transverse sections through this region reveals the presence of the anterior part of the trigeminal neural crest, which has become diffuse and has taken the form of trails of darkly-staining cells skirting beneath the somatic ectoderm (Pl. 1, fig. 15). Towards its caudal end the trigeminal neural crest remains more compact and is the anlage of the trigeminal ganglion (Text-figs. 4-6).

After the cells of the trigeminal neural crest have become scattered it is impossible to trace them, for their cytological appearance is not characteristic enough for their differentiation to be followed. There is, however, no evidence that they degenerate. Although the history of these neural crest cells cannot be followed there is at least no evidence to contradict the interpretation that they may behave in a similar manner to those of fishes and amphibia, where their differentiation can be demonstrated histologically and experimentally.

The epidermis overlying the area occupied by both diffuse and compact portions of the trigeminal neural crest corresponds with that of area V of Coërs (1946)—and it is this epidermis which shows placodal activity at a slightly later stage.

*(c) The origin of the ophthalmic lobe and ramus*

There is no evidence in the cat of the formation of a separate ophthalmicus profundus ganglion, although Coërs (1946) describes the formation of a separate ophthalmic ganglion of placodal origin in the rabbit.

The ophthalmic ramus in the cat first appears lateral to the anterior cardinal vein at the 5 mm. stage, at which time it is diffuse and entirely cellular. The appearance of the ramus coincides with the period of maximum placodal activity in area V. Nodules and strands of cells pass across from the epidermis and appear to cluster against the lateral wall of the main head vein. It is also possible that cells derived from the diffuse anterior portion of the trigeminal neural crest nebulize in this region, but the ophthalmic ramus appears to be derived at least in part from lateral head ectoderm. The ophthalmic lobe and ramus continue to receive epidermal contributions until the embryo attains a C.R. length of 8–9 mm. (Pl. 1, figs. 17, 18).

*(d) The formation of the trigeminal ganglion*

The anlage of the trigeminal ganglion is first derived from the posterior part of the trigeminal neural crest and can be identified from the 9-somite stage onwards (Text-figs. 1–10). By the 5 mm. stage there are signs of cellular degeneration within the trigeminal anlage. At this time cells from the epidermis of 'area V' overlying the ganglionic primordium are travelling across the intervening mesenchyme and passing into the trigeminal ganglion (Pl. 1, fig. 16). This process continues until the 10 mm. stage. As soon as the placodal cells are incorporated within the trigeminal anlage they cannot be distinguished from the neural crest cells. Hence, the pycnotic nuclear fragments found within the ganglion may be arising from the degeneration of either neural crest or placodal cells.

The histological evidence in the cat supports the view that the trigeminal ganglion receives during its establishment cells from two sources, namely the trigeminal neural crest and the lateral head ectoderm. But degeneration may overtake either type of cell once it is within the ganglion: hence it is very difficult to estimate what proportion of the neuroblasts of the trigeminal ganglion arise from neural crest and what proportion from lateral head ectoderm.

*B. Acoustico-facial neural crest*

*(a) Formation of acoustico-facial neural crest*

The acoustico-facial neural crest is first seen at the 8-somite stage. It lies at the anterior end of the otic placode and is to some extent over-lapped by it (Text-fig. 4). Anterior and posterior to this crest there is a length of neural tube which is devoid of any sign of neural crest formation.

The acoustico-facial neural crest continues to receive cells from the neural and juxta-neural ectoderm until at least the 20-somite stage. By the 14-somite stage the crest has extended ventro-laterally and is continuous with and indistinguishable

from the mesoderm of the hyoid arch, a condition which is even more striking at the 20-somite stage. Up to this time there are no signs of cellular degeneration in the acoustico-facial crest.

*(b) The fate of the acoustico-facial neural crest*

In 5 mm. embryos intense degeneration is taking place amongst the neural crest cells near the dorsal end of the hyoid arch. The dense column of cells which extended continuously from the neuro-somatic junction right down into the hyoid arch at the 20-somite stage is now interrupted by a zone that is becoming less dense and contains numerous pycnotic nuclear fragments. At the same time the epibranchial ectoderm of arch II is becoming placodally active; and cells are beginning to pass from this region into the underlying mesenchyme (Pl. 2, fig. 19; Text-fig. 9). This is the epibranchial placode of the facial nerve, and is similar to that described by Landacre (1932) in the rat.

The proximal part of the acoustico-facial crest at the 5 mm. stage is in contact with both the wall of the rhombencephalon and the antero-ventral wall of the otic vesicle. From both these sources cells are passing into the neural crest, while concurrently some signs of cellular degeneration can be detected within the cell mass. To what extent the neural crest cells remain to form neuroblasts, or to what extent they degenerate and are replaced by cells from the otic vesicle it is difficult to estimate. The migration of cells from the brain wall at the 5 mm. stage may be regarded as a tardy separation of neural crest material.

The distal portion of the acoustico-facial crest which extends down into the hyoid arch shows no sign of degeneration, and may develop into branchial arch cartilage.

*(c) Development of the geniculate ganglion*

The proliferation from the epibranchial ectoderm of the hyoid arch begins at the 5 mm. stage, before the geniculate ganglion is apparent in this region (Pl. 2, fig. 19). This diffuse mass of placodal cells extends towards the proximal portion of the acoustico-facial neural crest, and the two cell masses appear to mingle. During the 6-8 mm. stages a large epithelial spur extends from the epibranchial ectoderm into the proximal part of the acoustico-facial neural crest. Within this spur cells appear to be 'piling-up' on the epidermal side and then merging with the neuroblasts of the developing geniculate ganglion (Pl. 2, fig. 20). The geniculate ganglion is not clearly delimited dorsally from the auditory ganglion at the 6-8 mm. stage. At the 9 mm. stage the geniculate ganglion, consisting entirely of typical neuroblasts, has lost contact with its epibranchial placode and is becoming more clearly delimited from the developing acoustic ganglion. Thus, in the cat, the geniculate ganglion arises at least in part from the epibranchial placode, and possibly in part from the acoustico-facial neural crest.

*(d) Development of the auditory ganglia*

From the 5 mm. stage onwards an active migration of cells from the antero-ventral wall of the auditory vesicle is taking place (Pl. 2, fig. 21). These cells mingle with those of the proximal part of the acoustico-facial neural crest, and cannot be distinguished from them. The nuclear fragments found occasionally in this mixed



mass may be arising from degeneration of cells from either source. The cells from the auditory vesicle, even if not entirely responsible for the formation of the auditory ganglion, do at least contribute liberally towards it. When, at the 10 mm. stage, the acoustic ganglionic mass is differentiating into cochlear and vestibular primordia, the vestibular portion is still receiving numerous small, darkly-staining cells from the wall of the auditory vesicle. The auditory vesicle is the remaining portion of the acoustico-lateralis system in the mammal. The otic placode is a true dorso-lateral placode forming both sense organ and ganglion cells. Coërs (1946) has described vestigial pre- and post-otic dorso-lateral placodes in the rabbit, but no evidence of these structures was found in the present series of cat embryos.

*C. Vago-glossopharyngeal neural crest*

*(a) The formation of the vago-glossopharyngeal neural crest*

Although the vago-glossopharyngeal neural crest is discernible at the 8-somite stage it does not become conspicuous until the 14-somite stage. Cells are contributed to this crest by both the neural tube and juxta-neural ectoderm. At the 20-somite stage cells are leaving the distal end of the vago-glossopharyngeal crest and passing down into the developing caudal branchial arches.

At first the neural crest material forms a common vago-glossopharyngeal mass, but by the 5 mm. stage separate vagal and glossopharyngeal portions have been differentiated (Text-figs. 7, 8).

*(b) The development of the glossopharyngeal and vagal ganglia in relation to the cervical sinus*

After the cells of the ventral part of the glossopharyngeal crest have passed as mesectoderm into the third pharyngeal arch, the remaining mass of neural crest material subdivides into proximal and distal portions. The proximal part remains close to the brain wall and eventually forms the small superior ganglion of nerve IX. The distal part extends ventrally towards the third pharyngeal arch, from the dorsal end of which a mass of placodal cells is being proliferated. The proximal neural crest, distal neural crest and placodal proliferation can be seen as three cell masses in Pl. 2, fig. 22. The distal, or petrosal, neural crest and the epibranchial proliferation have fused by the 7 mm. stage to form the petrosal ganglion. While contact with the epidermis is maintained the cells at the distal end of the petrosal ganglion are small and irregular in shape and stain less intensely than the typical neuroblasts seen in the proximal part of the ganglion (Pl. 2, fig. 23). As soon as the petrosal ganglion loses contact with the epibranchial ectoderm the small placodal cells at the distal end cannot be distinguished from the neuroblasts of the more dorsal part of the ganglion.

At the 5 mm. stage the placode of the petrosal ganglion is situated at the dorsal end of the third pharyngeal arch just caudal to the closing plate between the second pouch and cleft (Text-figs. 8, 9).

By the 7 mm. stage the caudal end of the hyoid arch has overlapped the closing plate between the second pouch and cleft, but the ectodermal area of the third arch in contact with the petrosal ganglion is still exposed to the surface (Text-figs. 10, 11).

Continued growth of the hinder end of the hyoid arch causes further overlapping of the anterior part of the third arch, and by the 9 mm. stage the placode of the petrosal ganglion is hidden from the surface (Text-fig. 12). The ectoderm of the overhanging hyoid arch meets and fuses with the ectoderm of the third arch beneath it. In this process the closing plate of the second pouch and cleft is compressed into a solid column of cells, which at the 10 mm. stage bears the persisting contact of the petrosal ganglion (Text-fig. 13). By this time the contact of the petrosal ganglion with the epidermis is very slender and soon disappears; but as long as it remains the cells at the distal end of the ganglion are small and rounded. As soon as the placodal contact is lost the distinction between the small placodal cells and the neuroblasts is no longer apparent. A substantial part of the petrosal ganglion is evidently derived from the epibranchial placode.

The more caudal pharyngeal arches of the mammal are vestigial, and as a result of the formation of the cervical flexure become crowded into the sunken triangular area which is the cervical sinus (Frazer, 1925). The caudal border of the cervical sinus represents the dorsal ends of the suppressed caudal branchial arches; that is the epibranchial ectoderm of pharyngeal arches IV–VI. From this epibranchial ectoderm cells are proliferated into the underlying mesenchyme at the 5 mm. stage (Text-fig. 9). As the vagal neural crest subdivides into jugular and nodose portions the distal, or nodose part fuses with the proliferating epidermis at the caudal border of the cervical sinus (Text-figs. 9, 11). This proliferating epidermis is called the epibranchial placode of the vagus or the epibranchial placode of the fourth pharyngeal arch. In fact it represents the fused placodes of arches IV–VI whose separate appearance has been suppressed.

The histological appearance of the nodose ganglion while it remains in contact with the epibranchial ectoderm is similar to that of the petrosal described above: that is, it is composed of elongated neuroblasts proximally and small round cells distally. The proximal portion of the vagal neural crest remains close to the brain and forms the jugular ganglion.

When the vagus placode appears in 5 mm. embryos it is quite clearly an epibranchial placode (Text-fig. 9). Growth changes in the region of the cervical sinus tend, later, to obscure this fact. But it is the persistence of this placode, and that of the petrosal ganglion of nerve IX, which exerts a profound influence on the general anatomical changes in the region of the cervical sinus. As the placodal epidermis is anchored to the underlying petrosal and nodose ganglia it is not free to participate in the widening of the head, and tends to become buried beneath the surface. This may also prolong the duration of contact between the endodermal pouches and ectodermal clefts in the neighbourhood.

By the 7 mm. stage, when all four pharyngeal pouches are in contact with the ectoderm, the third and fourth arches appear relatively smaller than in 5 mm. embryos. This is due to the enlargement of the hyoid arch and to the growth of the tissue around the caudal border of the cervical sinus. The placode of the fourth arch, firmly united with the nodose ganglion becomes submerged beneath the general level of the epidermis (Text-fig. 11).

Further expansion of the head produces a pit at the bottom of which the vagus placode is situated at the 9 mm. stage (Pl. 2, fig. 24; Text-fig. 12). The closing plate

of the fourth pouch is carried into the pit during this process, but the closing plate of the third pouch still remains on the surface.

In embryos of 10 mm. the pit leading to the vagus placode is even deeper, and takes the form of a long narrow tunnel (Text-fig. 13). The fourth pouch has lost contact with the ectoderm and has become almost solid. The contact of the third pouch with its cleft has become absorbed into the anterior wall of the tunnel.

From the 5 to 10 mm. stages the epibranchial placode of the vagus is actively proliferating. In 11 mm. embryos the opening of the tunnel seals, thus forming a closed vesicle connected to the surface by a solid strand of epithelial cells. This vesicle is embedded in the distal end of the nodose ganglion, and its proliferative activity has waned. By the 12 mm. stage it loses contact with the nodose ganglion and remains as a tiny vesicle on the dorsal side of the third pouch; the latter having remained in contact with the ectoderm throughout is by now an almost solid epithelial mass.

#### SUMMARY

1. The cranial neural crest of the cat arises as three distinct masses—the trigeminal, the acoustico-facial and the vago-glossopharyngeal crest.

2. The anterior portion of the trigeminal crest dissociates as mesectoderm, while the posterior portion remains relatively compact and forms the anlage of the trigeminal ganglion. The ectoderm overlying the trigeminal crest becomes placodally active. Nodules and strands of epithelial cells detach from the epidermis and become incorporated within the trigeminal ganglion.

3. The acoustico-facial crest extends as a solid column of cells into the hyoid arch. Subsequently many of the crest cells in the epibranchial region degenerate, and the geniculate ganglion appears to arise in large part from an epibranchial placode. The auditory ganglion arises, at least in part if not entirely, from the epithelium of the auditory vesicle.

4. The vago-glossopharyngeal crest differentiates into separate glossopharyngeal and vagal portions. Cells become detached from the distal ends of both vagal and glossopharyngeal crests and appear to contribute to the tissue of the more posterior branchial arches.

The glossopharyngeal neural crest separates into superior and petrosal parts, and the petrosal portion fuses with an epithelial mass derived from the epibranchial ectoderm of arch III to form the petrosal ganglion.

The vagal neural crest forms separate jugular and nodose portions. The nodose neural crest fuses with the epibranchial ectoderm of the dorso-caudal border of the cervical sinus, and the nodose ganglion arises at the site of this fusion. This epibranchial ectoderm is the placode of the vagus nerve, and represents the fused epibranchial placodes of the more caudal pharyngeal arches. Later, the placode of the vagus becomes submerged beneath the surface ectoderm and soon separates from it as a small vesicle. This vesicle eventually loses contact with the nodose ganglion but remains attached to the dorsal side of the third pharyngeal pouch.



## LIST OF ABBREVIATIONS

<i>A I</i>	Pharyngeal arch I.	<i>VIII g.</i>	Auditory ganglion.
<i>A I (max.)</i>	Maxillary bud of arch I.	<i>VIII pl.</i>	Placode of auditory ganglion.
<i>A I (mand.)</i>	Mandibular rudiment.	<i>IX pet. g.</i>	Petrosal ganglion of glosso-pharyngeal.
<i>A II</i>	Pharyngeal arch II.	<i>IX sup. g.</i>	Superior ganglion of glosso-pharyngeal.
<i>A III</i>	Pharyngeal arch III.	<i>IX pl.</i>	Placode of petrosal ganglion.
<i>A IV</i>	Pharyngeal arch IV.	<i>X nod. g.</i>	Nodose ganglion of vagus.
<i>Ant. body fold</i>	Anterior body fold.	<i>X jug. g.</i>	Jugular ganglion of vagus.
<i>Ant. int. port.</i>	Anterior intestinal portal.	<i>X pl.</i>	Placode of nodose ganglion of vagus.
<i>Cl. plate</i>	Closing plate.	<i>Nodule</i>	Nodule of epidermal cells.
<i>Lens</i>	Lens.	<i>Op. ves.</i>	Optic vesicle.
<i>Mes.</i>	Mesencephalic flexure.	<i>Ot. pl.</i>	Otic placode.
<i>Nas. pl.</i>	Nasal placode.	<i>Ot. rh.</i>	Otic rhombomere.
<i>N.C. V</i>	Trigeminal neural crest.	<i>Ot. ves.</i>	Otic vesicle.
<i>N.C. V (dif.)</i>	Diffuse anterior portion of neural crest V.	<i>P 1</i>	First pharyngeal pouch.
<i>N.C. V (trig.)</i>	Compact posterior portion of neural crest V.	<i>P 2</i>	Second pharyngeal pouch.
<i>N.C. VII-VIII</i>	Acoustico-facial neural crest	<i>P 3</i>	Third pharyngeal pouch.
<i>N.C. IX-X</i>	Vago-glossopharyngeal neural crest.	<i>P 4</i>	Fourth pharyngeal pouch.
<i>N.C. IX</i>	Glossopharyngeal neural crest.	<i>Pl. tunnel</i>	Placodal tunnel.
<i>N.C. IX pet.</i>	Petrosal neural crest.	<i>Post-ot. s.</i>	Post-otic sulcus.
<i>N.C. IX sup.</i>	Superior portion of glosso-pharyngeal crest	<i>Pre-ot. s.</i>	Pre-otic sulcus.
<i>N.C. X</i>	Vagal neural crest	<i>Ros. flex.</i>	Rostral flexure.
<i>N.C. spin.</i>	Spinal neural crest.	<i>Som. I</i>	First somite.
<i>V trig. g.</i>	Trigeminal ganglion.	<i>Strand</i>	Strand of epidermal cells.
<i>V pl.</i>	Trigeminal placode.	<i>x</i>	Point of migration of cells from brain wall.
<i>VII g.</i>	Geniculate ganglion.	<i>y</i>	Point of migration of cells from otic placode.
<i>VII pl.</i>	Placode of geniculate ganglion.		

This investigation was carried out in the preparation of a thesis submitted for the London Ph.D. degree, under the supervision of Professor E. C. Amoroso, who gave freely of his advice and guidance throughout, and to whom I wish to express my thanks. Valuable help and advice was also given by my late colleague, Dr C. B. Murray. I am also grateful to Mr H. Burgess and the late Mr F. J. Pittock for taking many of the photographs. To Dr E. H. Batten I would like to express my thanks for his criticism and advice during the preparation of the abridged manuscript. A generous contribution towards the cost of publication of the photographic illustrations was received from the Colston Research Society of the University of Bristol.

# REFERENCES

- ADELMANN, H. B. (1925). The development of the neural folds and cranial ganglia of the rat. *J. comp. Neurol.* **39**, 19-123.
- BARTELMEZ, G. W. (1924). Ectodermal areas of the head in young human embryos. *Anat. Rec.* **29**, 109.
- BARTELMEZ, G. W. & EVANS, H. M. (1926). The development of the human embryo during the period of somite formation, including embryos with 2-16 pairs of somites. *Contr. Embryol. Carneg. Instn*, **17**, 1-67.
- BAXTER, J. S. & BOYD, J. D. (1939). Observations on the neural crest of a ten-somite human embryo. *J. Anat., Lond.*, **73**, 318-326.
- CAMPENHOUT, E. VAN (1935*a*). Origine du ganglion acoustique chez le porc. *Arch. Biol., Paris*, **46**, 271-285.
- CAMPENHOUT, E. VAN (1935*b*). Sur l'origine des ganglion craniens chez le porc et chez le poulet. *C.R. Soc. Biol., Paris*, **118**, 1653-1654.
- CAMPENHOUT, E. VAN (1936). Contribution à l'étude de l'origine des ganglions des nerfs craniens mixtes chez le porc. *Arch. Biol., Paris*, **47**, 585-604.
- CAMPENHOUT, E. VAN (1937*a*). Le rôle de placodes épiblastiques au cours du développement embryonnaire du porc et du poulet. *Bull. Acad. Méd. Belg.* **6**, 169-184.
- CAMPENHOUT, E. VAN (1937*b*). Le développement du système nerveux craniens chez le poulet. *Arch. Biol., Paris*, **48**, 611-666.
- CAMPENHOUT, E. VAN (1948). La contribution des placodes épiblastiques au développement des ganglions des nerfs craniens chez l'embryon humain. *Arch. Biol., Paris*, **59**, 253-266.
- COËRS, C. (1946). La formation des nerfs mixtes craniens chez le lapin. *Arch. Biol., Paris*, **57**, 13-79.
- DA COSTA, C. (1921). La crête ganglionnaire chez le cobaye. *C.R. Ass. Anat.* **16**, 173-178.
- DA COSTA, C. (1931). Sur la constitution et le développement des ébauches ganglionnaires craniennes chez les mammifères. *Arch. Biol., Paris*, **42**, 71-105.
- FRAZER, J. E. (1925). The disappearance of the precervical sinus. *J. Anat., Lond.*, **61**, 132-143.
- HOLMDAHL, D. E. (1928). Die Entstehung und weitere Entwicklung der Neuralleiste (Ganglienleiste) bei Vögeln und Säugetieren. *Z. mikr.-anat. Forsch.* **14**, 99-298.
- KAPPERS, A. (1941). Kopfplacoden bei Wirbeltieren. *Z. ges. Anat. 3. Ergebn. Anat. EntwGesch.* **33**, 371-412.
- KNOUFF, R. A. (1927). The origin of the cranial ganglia of *Rana*. *J. comp. Neurol.* **44**, 259-361.
- KNOUFF, R. A. (1935). The developmental pattern of ectodermal placodes in *Rana pipiens*. *J. comp. Neurol.* **62**, 17-65.
- KOSTIR, W. J. (1924). An analysis of the cranial ganglia of an embryo salamander, *Amblystoma Jeffersonianum*. *Ohio J. Sci.* **24**, no. 5.
- LANDACRE, F. (1908). The epibranchial placodes of *Ameiurus*. *Ohio Nat.* **8**, 251-255.
- LANDACRE, F. (1910*a*). The origin of the cranial ganglia in *Ameiurus*. *J. comp. Neurol.* **20**, 309-411.
- LANDACRE, F. (1910*b*). The origin of the sensory components of the cranial ganglia. *Anat. Rec.* **4**, 71-79.
- LANDACRE, F. (1912). The epibranchial placodes of *Lepidosteus osseus* and their relation to the cerebral ganglia. *J. comp. Neurol.* **22**, 1-69.
- LANDACRE, F. (1921). The fate of the neural crest in the head of the urodeles. *J. comp. Neurol.* **33**, 1-43.
- LANDACRE, F. (1927). The differentiation of the pre-auditory and post-auditory primitive lines into pre-auditory and post-auditory placodes, lateralis ganglia and migratory lateral line placodes in *Amblystoma Jeffersonianum*. *J. comp. Neurol.* **44**, 29-60.
- LANDACRE, F. (1931). The epibranchial ganglion of the glossopharyngeal nerve in *Amblystoma Jeffersonianum*. *Ohio J. Sci.* **31**, 335-345.
- LANDACRE, F. (1932). The epibranchial placode of the facial nerve in the rat. *J. comp. Neurol.* **56**, 215-255.
- LANDACRE, F. (1933). The epibranchial placode of the facial nerve in *Amblystoma Jeffersonianum*. *J. comp. Neurol.* **58**, 289-309.
- REED, C. I. (1916). The epibranchial placodes of *Squalus acanthias*. *Ohio J. Sci.* **16** (8), 336-354.
- STONE, L. S. (1922). Experiments on the development of the cranial ganglia and lateral line sense organs in *Amblystoma punctatum*. *J. exp. Zool.* **35**, 421-496.

- STONE, L. S. (1924). Experiments on the transplantation of placodes of the cranial ganglia in the amphibian embryo. I. Heterotopic transplantations of the ophthalmic placode upon the head of *Amblystoma punctatum*. *J. comp. Neurol.* **38**, 73-105.
- STONE, L. S. (1928a). Primitive lines in *Amblystoma* and their relation to the migratory lateral line primordia. *J. comp. Neurol.* **45**, 169-190.
- STONE, L. S. (1928b). Experiments on the transplantation of placodes of the cranial ganglia in the amphibian embryo. II. Heterotopic transplantation of the ophthalmic placode upon the head and body of *Amblystoma punctatum*. *J. comp. Neurol.* **47**, 61-116.
- STONE, L. S. (1928c). Experiments on the transplantation of placodes of the cranial ganglia in the amphibian embryo. III. Pre-auditory and post-auditory placodal materials interchanged. *J. comp. Neurol.* **47**, 117-154.
- STONE, L. S. (1929a). Experiments on the transplantation of placodes of the cranial ganglia in the amphibian embryo. IV. Heterotopic transplantation of the post-auditory placodal material upon the head and body of *Amblystoma punctatum*. *J. comp. Neurol.* **48**, 311-330.
- STONE, L. S. (1929b). Experiments showing the role of migrating neural crest (mesectoderm) in the formation of the head skeleton and loose connective tissue in *Rana palustris*. *Arch. Entom. Mech. Org.* **118**, 40-77.
- YNTEMA, C. L. (1944). Experiments on the origin of the sensory ganglia of the facial nerve in the chick. *J. comp. Neurol.* **81**, 147-163.

## EXPLANATION OF PLATES

### PLATE 1

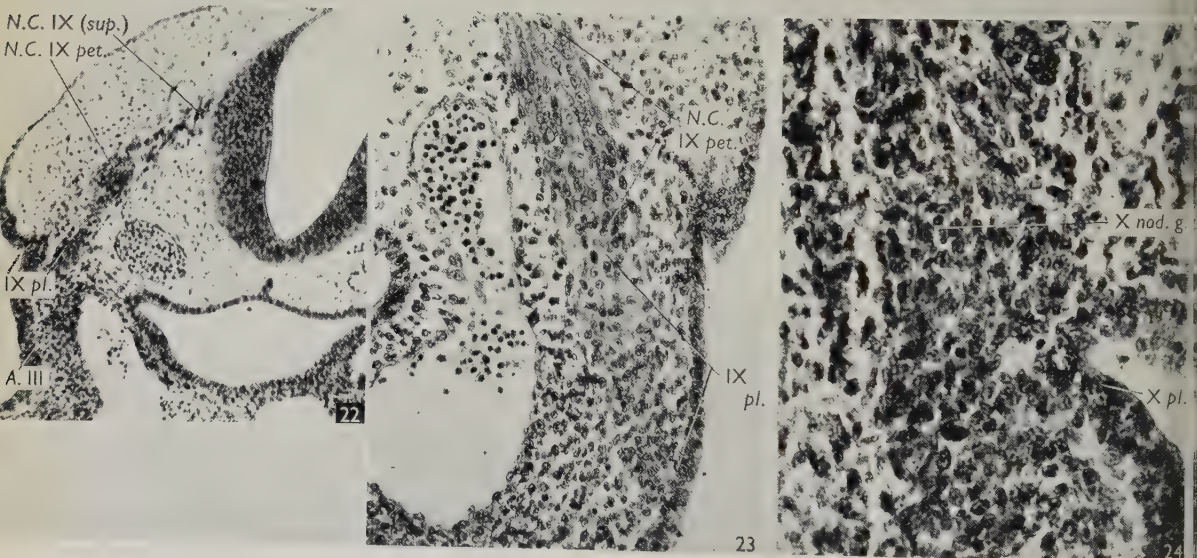
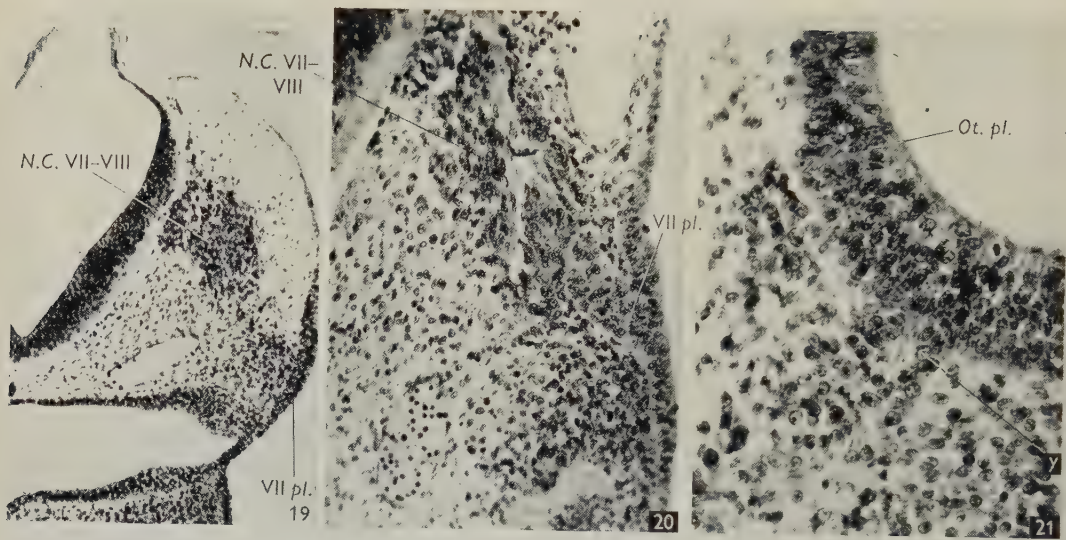
- Fig. 14. Transverse section through the trigeminal neural crest of a 7-somite cat embryo. See line X-Y in Text-fig. 3. ( $\times 104$ .)
- Fig. 15. Transverse section (slightly oblique) through an 8-somite cat embryo just caudal to the optic vesicles showing the diffuse anterior portion of the trigeminal neural crest. See line X-Y on Text-fig. 4. ( $\times 97$ .)
- Fig. 16. Coronal section of 5 mm. cat embryo in the region of the trigeminal ganglion. Mesectoderm still appears to be arising from the distal end of the crest. The somatic ectoderm overlying this neural crest is actively proliferating cells into the underlying mesenchyme at this stage. ( $\times 78$ .)
- Fig. 17. Section through the trigeminal ganglion of a 7 mm. cat embryo showing a strand of cells which has separated from the overlying epidermis. ( $\times 453$ .)
- Fig. 18. Section through the trigeminal ganglion of a 7 mm. cat embryo showing a nodule of cells becoming detached from the placodal epidermis overlying the ganglion. ( $\times 415$ .)

### PLATE 2

- Fig. 19. Coronal section through 5 mm. cat embryo in the region of the acoustico-facial neural crest and epi-branchial placode of the facial nerve. ( $\times 84$ .)
- Fig. 20. Coronal section through head of 7 mm. cat embryo in the region of the epibranchial placode of the facial nerve. A large placodal spur passes into the substance of the ganglion. ( $\times 150$ .)
- Fig. 21. Transverse section through head of 8 mm. cat embryo in the region of the otic vesicle and auditory ganglion. At Y cells can be seen streaming out of the otic epithelium into the ganglion. ( $\times 300$ .)
- Fig. 22. Coronal section of 5 mm. cat embryo showing the superior and petrosal portions of the glossopharyngeal neural crest. The section also passes through the epibranchial placode of the petrosal ganglion. ( $\times 95$ .)
- Fig. 23. Coronal section of 8 mm. cat embryo in the region of the petrosal ganglion. The placodal proliferation and petrosal neural crest (seen separately in Fig. 22) have fused, but remain histologically distinct. ( $\times 174$ .)
- Fig. 24. Coronal section of 9 mm. cat embryo showing the epibranchial placode of the nodose ganglion of the vagus. The placode lies at the bottom of a pit. ( $\times 270$ .)







MALLEY—THE PLACODAL RELATIONS OF THE NEURAL CREST IN THE DOMESTIC CAT



# CELL DEGENERATION DURING NORMAL ONTOGENESIS OF THE RABBIT BRAIN

By BENGT KÄLLÉN\*

*Department of Anatomy, University College, London*

## INTRODUCTION

Cell degeneration during normal ontogenesis in vertebrates has been described many times (Glücksman, 1951). Few observations have been made on such processes in the central nervous system after the stage of formation of the neural tube. Ernst (1926) described cell degeneration in the floor of the third and fourth ventricles, in the optic evaginations and surrounding parts of the brain wall and, in mammals, in the paraphyseal rudiment. Graumann (1950) discussed the 'paraphyseal degeneration' in the mouse and thought it to be related to the formation of commissures. In the spinal cord degeneration has been described by Ernst (1926), Froboese (1926), Glücksman (1930), Hamburger & Levi-Montalcini (1950) and Hamburger (1952).

The earlier stages in the development of the nervous system are better known from this point of view. The most detailed description is given by Elwood (1951) in an unpublished thesis. He described two peaks of degeneration, one earlier that he thinks is related to the differentiation from ectodermal cell to neural plate cell, and one later, which he thinks is related to the rolling up of the plate to form a tube. Several authors (cf. Glücksman, 1951) have described cell death at the region of separation of the neural tube from the surface ectoderm, especially in the neighbourhood of the rostral neuropore. This paper consists of a systematic description of cell degeneration in the brain of a mammal. Such a description seems to be a necessary basis for any interpretation of the significance of this process in the developmental mechanism of the neural tube.

## MATERIAL AND METHODS

The investigation was carried out on rabbit embryos, belonging to the J. P. Hill Embryological Collection at University College, London. The embryos studied are summarized in Table 1. Graphical reconstructions were made in some cases (see the table), and regions with concentrations of cell degeneration were marked. All embryos were cut transversely and stained with haematoxylin-eosin. In some older embryos where no centres of cell deaths exist, quantitative calculations were also made in the following way: the total nuclear number and the number of degeneration nuclei were counted under high magnification (oil-immersion objective,  $\times 100$ ; ocular  $\times 10$ ) with the help of a squared graticule in the ocular. The counts were made on four sections from each embryo, one from the diencephalon, one from the mesencephalon and two from the rhombencephalon. In each section four different microscopic fields were counted. When a peripheral layer was present, four fields were counted in it and four in the ventricular layer. As the section

\* On leave from Tornblad Institute, Lund, Sweden. The investigation was made possible by a grant from the Swedish Medical Research Council.



thickness was a little different in the different series used, the numbers obtained were corrected according to the formula given by Abercrombie (1946). The diameters of the normal and degenerating nuclei were determined by measurement with an ocular millimetre scale. No significant variation in nuclear diameter between the different stages was found. The mean diameter of the normal nucleus was  $5.2\mu$ , the diameter of the degenerating nucleus  $3.5\mu$ .

Table 1. *Data on embryos investigated*

(Specimen number refers to the University College collection.)

No.	No. of somites	Age		Length (mm.)	Thickness of sections ( $\mu$ )	Reconstruction
		(days)	(hr.)			
R 200	13	9	0	—	10	G $\times$ 100
R 24.7.11	21	9	16	—	8	G $\times$ 100
						W $\times$ 125
R 247	23	9	16	—	8	G $\times$ 100
R 246	27-28	9	16	—	8	G $\times$ 100
R 279	31-32	10	18	—	8	—
R 11 d6 mm	33	11	0	6	8	G $\times$ 50
R(6) 11d	37	12	0	6	8	—
R7.7.21B	—	12	3	7	10	—
R7.7.21C	—	12	3	7	10	—
R226a	—	13	0	9.5	10	—
R432	—	14	0	10	10	—
R423	—	14	0	11.5	10	—
R422	—	14	0	11.5	10	G $\times$ 50
R209	—	15	0	14	12	—

W marks a wax-plate, G a graphical reconstruction of the brain.

## RESULTS

*Stage, 13 somites (R 200\*) (Text-fig. 1)*

The cerebral tube is closed except rostrally, where the anterior neuropore is still open. There are distinct proneuromeres present. The optic evaginations are relatively shallow bulges. On their caudal surface a very faint groove is marked, called *fiss. P 1* in Text fig. 1 and demarcating the first and second proneuromeres. The very poor development of the groove makes it probable that it is not a true proneuromeric fissure but only a result of the development of the optic evaginations. If this is the case the first and second proneuromeres are fused—a possibility discussed by Bergquist & Källén (1954). Two ganglion anlagen are present, a trigeminal anlage level with the fourth proneuromere and a facialis-acusticus one level with the fifth proneuromere.

Cell degeneration is found in the following regions:

- (1) In the dorsal edge of the anterior neuropore.
  - (2) Ventral to the anterior neuropore and to the optic evaginations.
  - (3) In the dorso-median parts of the second and third proneuromeres.
  - (4) In the median part rostral to the ependymal roof of the rhombencephalon.
- Besides these a very few scattered dying cells are found elsewhere in the brain.

*Stage, 21 somites (R 24. 7. 11) (Text-fig. 2)*

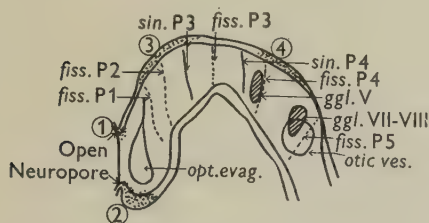
The stage is a transitory stage between the proneuromeric and the neuromeric phase. Rostrally there are distinct neuromeres present, but caudally there are

\* These slide references are to the specimens in collections at University College.

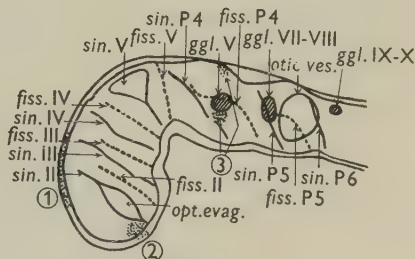
proneuromeres. The external fissures are here somewhat irregular. The optic evaginations are distinct but there are no signs of optic cups. A glossopharyngicus-vagus ganglion anlage is now also present.

Degenerating cells have been found in the following regions:

- (1) A few along the rostro-dorsal median line within neuromeres II and III.
- (2) A few ventro-rostral to the optic evaginations.
- (3) A few ventral to the trigeminal ganglion and a few in the dorsal part of the seventh neuromere.



Text-fig. 1.



Text-fig. 2.

Text-fig. 1. Graphical reconstruction of the brain of embryo R 200 in medial view. Ventricular furrows are marked with whole lines, the projections of the external fissures with dashes; the ganglionic anlagen are hatched. Regions containing degenerating cells are dotted. Magnification,  $\times 33$ .

Text-fig. 2. Graphical reconstruction of the brain of embryo R 24.7.11, in medial view. Designations as in Text-fig. 1. Magnification,  $\times 33$ .

#### Stage, 23 somites (R 247) (Text-fig. 3)

This stage has eleven neuromeres clearly visible in the reconstruction. No optic cups are yet formed. Degenerating cells occur in the following parts:

- (1) In the dorso-median part of the four first neuromeres (II-V).
- (2) Many rostro-ventral to the optic evaginations.
- (3) A few in the ventro-median part of the fourth neuromere.
- (4) Ventral to the trigeminal ganglion and dorsally in the seventh neuromere.

#### Stage, 27-28 somites (R 246) (Text-fig. 4 and Pl. 1, figs. 1-3)

The stage is a late neuromeric stage and the rhombomeres are not clearly seen except the seventh neuromere, which forms a distinct furrow (*sin. VII*) level with the trigeminal ganglion. No optic cups are yet formed.

Degenerating cells are found in the following places:

- (1) Along the whole of the dorso-median line rostral to the middle of the fifth neuromere.

(2) In the lateral wall around the dorsal part of *fiss. IV* (Pl. 1, fig. 2).

(3) Ventral to the optic evaginations (Pl. 1, fig. 3).

(4) Level with the trigeminal ganglion (in neuromere VII) (Pl. 1, fig. 1).

(5) Level with or just rostral to the facialis-acusticus ganglion (in neuromere IX).

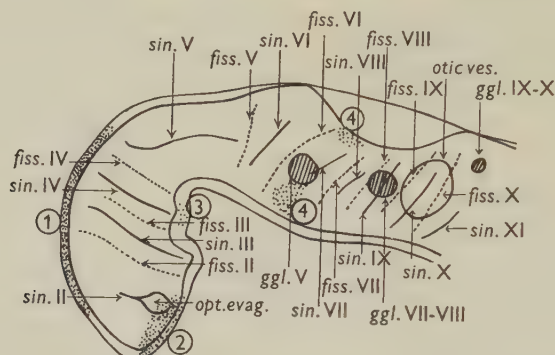
(6) Just rostral to the glossopharyngicus-vagus ganglion (in neuromere XI).

*Stage, 31-32 somites (R 279)*

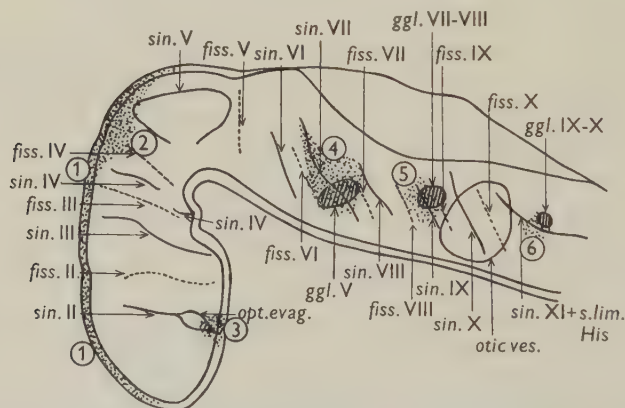
A few degenerating cells are seen in the dorso-median part of the archencephalon. Ventral to the optic evaginations there is much cell degeneration. A few scattered dying cells are found in the rhombencephalon.

*Stage, 33 somites (R11 d6 mm) (Text-fig. 5)*

This stage shows early migration areas (cf. Bergquist & Källén, 1954). The cell migration is poorly marked but the transverse furrows present show that the 'transversal bands' of the areas have been formed, at least rostrally. A distinct hemispheric evagination is now visible, and the optic cups have appeared.



Text-fig. 3. Graphical reconstruction of the brain of embryo R 247, in medial view. Designations as in Text-fig. 1. Magnification,  $\times 33$ .



Text-fig. 4. Graphical reconstruction of the brain of embryo R 246, in medial view. Designations as in Text-fig. 1. Magnification,  $\times 33$ .

Degenerating cells are found:

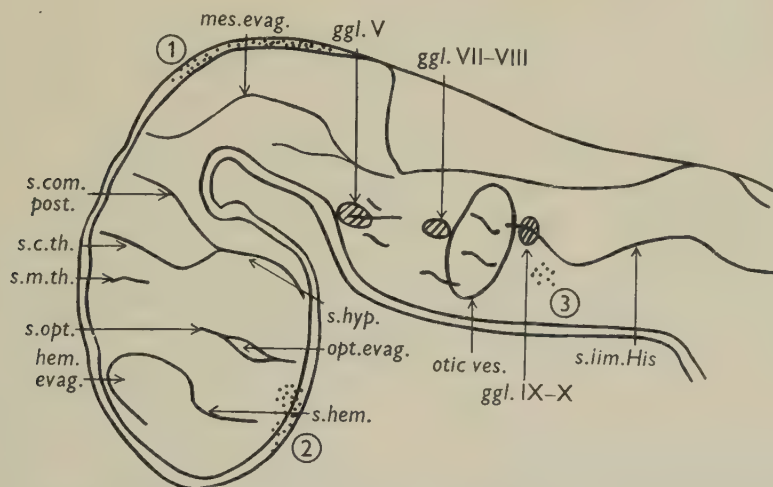
- (1) A few in the dorso-median part of the mesencephalon.
- (2) A few (chiefly in the median plane) rostral to the optic evaginations.
- (3) A few scattered in the rhombencephalon, possibly a little more commonly at the level of the glossopharyngeal-vagus ganglion.



## Stage, 12 days, 3 hr. (R 7.7.21 B)

This stage has migration areas with a marked migration of cells. There are very deep and distinct transverse furrows present in the rhombencephalon.

Degenerating cells are found scattered in the brain, but no distinct aggregations of them are found, even around the optic stalks.



Text-fig. 5. Graphical reconstruction of the brain of embryo R 11 d6 mm, in medial view. Designations as in Text-fig. 1. Magnification,  $\times 25$ .

Table 2. Frequency of degenerating cells as a proportion of all brain cells in embryos of different ages

No.	Age (days) (hr.)	Dying cells as a percentage of total cell number	
		Ventricularly	Peripherally
R 11 d6 mm	11 0		0.81*
R(6) 11 d	11 0		0.59
R 7.7.21 B	12 3		0.46
R 7.7.21 C	12 3		0.69
R 226a	13 0		0.63
R 432	14 0	6.19	6.03
R 423	14 0	2.61	10.20
R 422	14 0	2.99	16.39
R 209	15 0	0.90	0.83

\* As is apparent from the description in the text, there are small death centres in this stage. They have, however, been avoided at the choice of the sections for the count.

In older stages the same condition exists, no distinct centres of cell death are visible.

Some calculations have been made in order to compare the intensity of cell degeneration in different stages. The number of visible degenerating cells was calculated as a percentage of the total number of cells in certain stages and the results are given in Table 2.

The 'death-rate' is low in all stages except the three 14-day embryos. The mean of the embryos other than 14-day ones, is 0.70 with a standard deviation of the

distribution of 0-15. The rate in the 14-day stages is obviously significantly higher. In stage R 432 the rate is lower than in the other two and equally large in the ventricular and peripheral part. These two parts are, however, not yet distinctly separated. In the other two stages the rate is higher in the peripheral than in the ventricular part. In order to see if this difference is significant, the rate was determined at thirteen different points, scattered all over the brain, in series R 422.\* The results of this are shown in Table 3.

The values of the rates in the peripheral layer vary, but in every situation they are conspicuously above the corresponding values in the ventricular part.

#### DISCUSSION

It is thus apparent that from the point of view of cell degeneration two main periods in the development of the brain can be distinguished.

A. An early phase extending from the time of formation of the neural tube up to about the 11th day of gestation (i.e. approximately up to and including the neuro-

Table 3. *Comparison of frequency of degenerating cells in the ventricular and peripheral parts of various regions of the brain and spinal cord*

Place	Dying cells as a percentage of total cell number	
	Ventricularly	Peripherally
Rostral part of the hemisphere	1.3	12.8
Caudal part of the hemisphere	0.9	21.8
Preoptic region	1.4	8.8
Mammillary region	2.1	27.4
Thalamic region	1.5	17.1
Region of the area fasciculi longitudinalis medialis	1.7	6.5
Rostral part of the mesencephalon	2.4	7.1
Caudal part of the mesencephalon	1.1	9.7
Isthmic region	1.9	11.8
Rostral part of rhombencephalon	5.1	28.9
Middle part of rhombencephalon	4.5	7.8
Caudal part of rhombencephalon	1.7	6.5
Rostral end of spinal cord	2.0	12.3

meric phase). In this period degenerating cells are found aggregated in centres, which are most developed in the 27-28-somite stage and are discussed in detail below.

B. A later phase extending from the 11th day of gestation and thus including the stages of migration areas and later stages of nuclear differentiation. In this phase degenerating cells are found scattered all over the brain. The death-rate is low except in the 14th day where a steep increase occurs.

The various centres of degeneration of the first phase will now be discussed. In the 13-somite stage degenerating cells were found at the anterior neuropore which is still open. They seem to be a remnant of the separation process of the neural tube from the surface ectoderm, which is characterized by much cell death, as described by Glücksmann and others. In all embryos up to and including the 11-day stage dying cells were also found in the dorso-median part of the archencephalon. These

\* Here the same area in four consecutive sections has been counted. As the thickness of the sections and the nuclear diameters are the same in all these counts, no correction by Abercrombie's (1946) formula is necessary.

might also be interpreted as remnants of the separation process but this seems unlikely as they increase in number and extent up to the 27–28-somite stage and then decrease again, so that very few are left in the 33-somite stage and after that have all disappeared. They resemble part of the degenerating cells described by Graumann (1950). It seems likely that they represent a 'morphogenetic degeneration' related to the formation of the tela chorioidea ventriculi tertii and related structures and, in the mesencephalon, of the dorsal raphe. Similarly situated cells in the rostral part of the rhombencephalon in the 13-somite stage probably take part in the formation of the tela chorioidea ventriculi quartii.

In all the embryos younger than 12 days a distinct 'death centre' is found ventral and often rostral to the optic evaginations. This observation agrees with that of Ernst (1926). These cell deaths seem to be related to the formation of the chiasma system and might at least partly be responsible for the ventral shifting of the optic stalk.

In the lateral part of the brain wall in these young embryos other degenerating cells have been found in the dorsal part of the fourth and fifth neuromeres and in the rhombencephalon, level with the ganglia. The former cell degenerations seem to be related to the dorso-median cell death already discussed, and are only present when this is maximally developed.

The cell degeneration at the level of the ganglia is of special interest. The first of these dying cells to become visible lie level with the trigeminal ganglion, dorsal and ventral to its attachment to the neural tube. In the 27–28-somite stage they are situated mainly in the middle of the brain wall, just opposite the ganglion. In this stage similar 'death centres' are also present level with the other ganglia. They all disappear rapidly, and are hardly seen in the 33-somite stage. These dying cells seem to correspond to those described by Ernst in the rhombencephalon of, among others, rabbit embryos in the following way: 'Am ausgedehntesten und regelmässigen fand ich die Degeneration bei 12 Tage alten Embryonen am Boden des hinteren Abschnitte des IV. Ventrikels. Hier sind in der Lamina basalis auf eine grosse Strecke Gruppen von degenerierten Zellen zu finden, die meist in der Mitte der Wanddicke liegen. Sie bilden keine zusammenhängenden Schicht, sondern sind auf 4–5 Schnitten auf beiden Seiten deutlich, dann kommen ebenso viele, auf denen keine Degeneration zu sehen sind' (p. 243). Ernst, however, does not describe the typical positions of these centres of cell death.

Hamburger & Levi-Montalcini (1950) and Hamburger (1952) have described how cell death in the cervical region of the spinal cord in chick embryos causes the differences in thickness of the motor column. In the brain the regional differences are formed by the transverse proliferation patterns; no cell death is necessary for this purpose. The only unevenly distributed cell death in the rhombencephalon is that described above, and this should have the opposite effect to that described by Hamburger, as it *lessens* the cell number level with the ganglia, where the motor columns will later be thickest. This cell death thus seems to have some other function. It might represent the first signs of a 'histiogenetic degeneration' (see below) of phase B, its distribution being the result of a possible earlier start of differentiation level with the ganglia. Against this opinion, however, are the facts: (1) that the centres of cell death seem to disappear before the 'histiogenetic degeneration' starts.



They do not fuse but seem to dissolve. (2) That at least the rostral-most centre begins to develop dorsally and ventrally in the tube and not level with the point of attachment of the trigeminus ganglion crest. There is therefore at present no plausible explanation of these centres. It should be emphasized that the primary stimuli causing the areas of degeneration are unknown.

During phase B the death-rate is relatively low except in the 14-day stage, where a sharp increase takes place. This increase is greater in the peripheral part than in the ventricular part. These facts speak in favour of the opinion that it represents a 'histiogenetic degeneration' according to Glücksmann's terminology (1951). It takes place just at the time of the beginning of cell differentiation via neuroblast or spongioblast to neuron or glia cell and is stronger in the peripheral part, where also the differentiation processes are more marked. A detailed analysis of the regional differences in death-rate in closely placed stages might help to elucidate the mode of starting of the differentiation processes, an investigation that the author's material does not permit.

#### SUMMARY

1. Cell deaths have been studied in a series of rabbit embryos from the time of closing of the neural tube to the 15th day of gestation with the help of reconstructions and counts of the numbers of degenerating cells.

2. In stages earlier than 11 days dying cells are found in relatively well delimited centres. These are most conspicuous in the 27-28-somite stage. The function of some of these centres can be suggested but the primary stimuli causing them are still unknown.

3. In stages after the 11th day the death-rate is low except during the 14th day, where a high rate is found. Here the rate is higher in the peripheral than in the ventricular part of the brain and probably represents a 'histiogenetic degeneration'.

I want to take this opportunity to express my deep thanks to Prof. J. Z. Young and Mr M. Abercrombie for all the help and kindness they have shown me during my stay at University College.

#### REFERENCES

- ABERCROMBIE, M. (1946). Estimation of nuclear population from microtome sections. *Anat. Rec.* **94**, 239-247.
- BERGQUIST, H. & KÄLLÉN, B. (1954). Notes on the early histogenesis and morphogenesis of the central nervous system in vertebrates. *J. comp. Neurol.* **100**, 627-660.
- ELWOOD, M. A. (1951). Quantitative studies on the early development of the nervous system and associated structures. Thesis, University of London.
- ERNST, M. (1926). Ueber Untergang von Zellen während der normalen Entwicklung bei Wirbeltieren. *Z. ges. Anat. 1. Z. Anat. EntwGesch.* **79**, 228-262.
- FROBOESE, C. (1926). Das Vorkommen von Fett in Embryonen. *Z. mikr-anat. Forsch.* **7**, 527-671.
- GLÜCKSMANN, A. (1930). Ueber die Bedeutung von Zellvorgängen für die Formbildung epithelialer Organe. *Z. ges. Anat. 1. Z. Anat. EntwGesch.* **93**, 35-92.
- GLÜCKSMANN, A. (1951). Cell deaths in normal vertebrate ontogeny. *Biol. Rev.* **26**, 59-86.
- GRAUMANN, W. (1950). Zelldegeneration im Telencephalon medium und Paraphysenentwicklung bei der weissen Maus. *Z. ges. Anat. 1. Z. Anat. EntwGesch.* **115**, 19-31.
- HAMBURGER, V. (1952). Development of the nervous system. In: *The chick embryo in biological research*. *Ann. N.Y. Acad. Sci.* **55**, no. 2, 117-132.
- HAMBURGER, V. & LEVI-MONTALCINI, R. (1950). Some aspects of neuroembryology. Weiss, ed.: *Genetic Neurology*, pp. 128-160. The University of Chicago Press.







# ABBREVIATIONS

<i>fiss.</i> I-XII	fissura interneuromerica I-XII
<i>fiss.</i> P1-P5	fissura interproneuromerica 1-5
<i>ggl.</i> V	anlage of trigeminus ganglion
<i>ggl.</i> VII-VIII	anlage of facialis-acusticus ganglion
<i>ggl.</i> IX-X	anlage of glossopharyngicus-vagus ganglion
<i>hem.evag.</i>	hemispheric evagination
<i>mes.evag.</i>	mesencephalic evagination
<i>opt.evag.</i>	optic evagination
<i>otic ves.</i>	otic vesicle
<i>s.c.th.</i>	sulcus caudalis thalami
<i>s.com.post.</i>	sulcus commissurae posterioris
<i>s.hem.</i>	hemispheric groove
<i>s.hyp.</i>	sulcus hypothalamicus
<i>s.lim.His</i>	sulcus limitans His
<i>s.m.th.</i>	sulcus medialis thalami
<i>s.opt.</i>	optic groove
<i>sin.</i> I-XII	ventricular neuromeric furrows I-XII
<i>sin.</i> P1-P6	ventricular proneuromeric furrows 1-6
(1), (2) etc.	mark the 'death centres' and refer to the numbering in the description of the stages in the text.

## EXPLANATION OF PLATE 1

- Fig. 1. Transverse section of the brain wall in embryo R246. The figure shows the dorsal part of the rhombencephalon level with the trigeminus ganglion anlage and shows moderate cell degeneration in the brain wall. Magnification,  $\times 185$ .
- Fig. 2. Transverse section of the brain wall in embryo R246. The figure shows the part around the *fiss.* IV and shows much cell degeneration. Magnification,  $\times 185$ .
- Fig. 3. Transverse section of the brain wall in embryo R246. The figure shows the caudal part of the optic evagination with much cell degeneration. Magnification,  $\times 185$ .

## STUDIES ON THE INNERVATION OF SKIN

### I. THE ORIGIN, COURSE AND NUMBER OF SENSORY NERVES SUPPLYING THE RABBIT EAR

By G. WEDDELL, W. PALLIE AND ELIZABETH PALMER

*Department of Anatomy, University of Oxford*

#### INTRODUCTION

In recent papers (Lele, 1954; Lele, Weddell & Williams, 1954) evidence has been presented which suggests that the stimulation of unencapsulated nerve endings in hairy skin can elicit verbal reports which embrace a wide range of sensory experience, each report varying in accordance with the temporo-spatial pattern of the nerve impulses which the unencapsulated endings discharge on to the central nervous system. Unfortunately, we have not found it possible by histological methods to determine the precise anatomical arrangement which underlies the pattern of nerve impulses which are evoked when unencapsulated nerve terminals are stimulated. This is due to technical difficulties arising from the delicacy, density, proximity and wide area covered by the fine naked axoplasmic filaments which spring from ensheathed stem axons situated in all strata of the skin (Weddell, Pallie & Palmer, 1954).

We therefore decided to study the innervation of hairs on a quantitative basis to determine whether the anatomical arrangements were such that the stimulation of a hair in a specific manner was likely to give rise to a discharge of nerve impulses of a characteristic temporo-spatial pattern. The rabbit ear was selected for this investigation since Sinclair, Weddell & Zander (1952) and Weddell *et al.* (1954) have shown that in the skin of both rabbit and human ears there are no encapsulated nerve endings other than those related to hair follicles, each of which is associated with a number of myelinated nerve fibres terminating in fine, naked, freely ending axoplasmic filaments arranged in a complex but orderly fashion. Our observations fall naturally into three interdependent parts which we have assembled in the form of three separate but consecutive papers. The first paper is concerned with the origin, course, number and fibre size of the spinal and cranial sensory nerve fibres distributed to the rabbit ear. The second paper is concerned with the number, size and distribution of hairs, hair follicles and orifices in the skin from which the hairs emerge, and the third with the patterned arrangement of the nerve fibres supplying the ear.

The origin and course of the spinal and cranial sensory nerves which enter the rabbit ear was determined by dissection and degeneration experiments. The number of nerve fibres supplying the ear was determined by counting the number of individual nerve fibres in transverse sections from the main nerve trunks, alternate sections being stained for myelin and impregnated with silver respectively. The number of nerve fibres comprising the small branches (from the cranial nerves) was

determined after section, resection and degeneration of the main nerve trunks (from the spinal nerves) from appropriately stained horizontal sections through the base of the ear distal to the point of nerve section.

Grant, Bland & Camp (1932) have given the fullest description so far of the innervation of the rabbit ear. Briefly, they have shown that the ear can be completely denervated by section of the great auricular and lesser occipital (posterior auricular) nerves (derived from the second and third cervical nerve roots), the auriculo-temporal branch of the trigeminal nerve, the auricular branch of the vagus nerve and complete excision of the cervical sympathetic chain of the same side. On the basis of this information we proceeded as follows.

#### MATERIAL AND METHODS

In all, forty rabbits of Dutch or Copenhagen strain were used. The origin and course of the great auricular and lesser occipital nerves was determined by dissection. Fibre counts were made at many different positions along the course of these nerve trunks, but in later specimens we concentrated on the positions indicated in Fig. 1, *A* to *B*, *C* to *D*, and *E* to *F*. Counts were also made immediately proximal and distal to the second and third cervical dorsal root ganglia and directly after fusion of the anterior and posterior nerve roots. Segments of nerve for fibre counting were obtained from anaesthetized animals as follows: the nerves were exposed rapidly, flooded with a 0.025 % solution of Hyaluronidase ('Hyalase' Benger) and removed 20 min. later. Alternate sections from each segment were stained for myelin (Lison's method) and impregnated with silver (Romanes's method).

The auricular branch of the vagus and trigeminal nerves which enter the ear are small and difficult to dissect and to remove in an undamaged state for the purpose of making fibre counts. We therefore sectioned and resected 2 mm. of the great auricular and lesser occipital nerves proximal to the root of the ear in two rabbits. Fourteen days later the operated ears were removed and embedded in celloidin. Sections horizontal to the long axis of the ear were cut through the root of the ear just distal to the point of nerve section and alternate sections stained with myelin and impregnated with silver. The fibres in the intact nerve bundles were then counted.

In addition, in two rabbits, the course of (and approximate area of skin supplied by) the auricular branch of the vagus to the dorsum of the ear was determined by section and resection of 2 cm. of the great auricular and lesser occipital nerves at the root of the ear, and 2 weeks later staining the whole of the skin of the dorsum of the ear with methylene blue by the method described by Weddell & Pallie (1954). It was also possible to estimate approximately in these specimens the number of nerve fibres comprising the auricular branch of the vagus to the dorsum of the ear.

#### OBSERVATIONS

##### (1) *Topography of the spinal nerves*

The description which follows obtained in all the rabbits we dissected. Minor variations were encountered but none which invalidated the overall picture which we shall give. The precise point of origin, together with details of the size and course of



the smaller nerve bundles leaving and acceding to the main nerve trunks were, however, subject to variation from animal to animal.

The great auricular and lesser occipital nerves were traced to their origin from the second and third cervical roots. We confirmed that these were the only two cervical roots concerned with the formation of these nerves in the rabbits we dissected. The great auricular nerve receives its major contribution from the

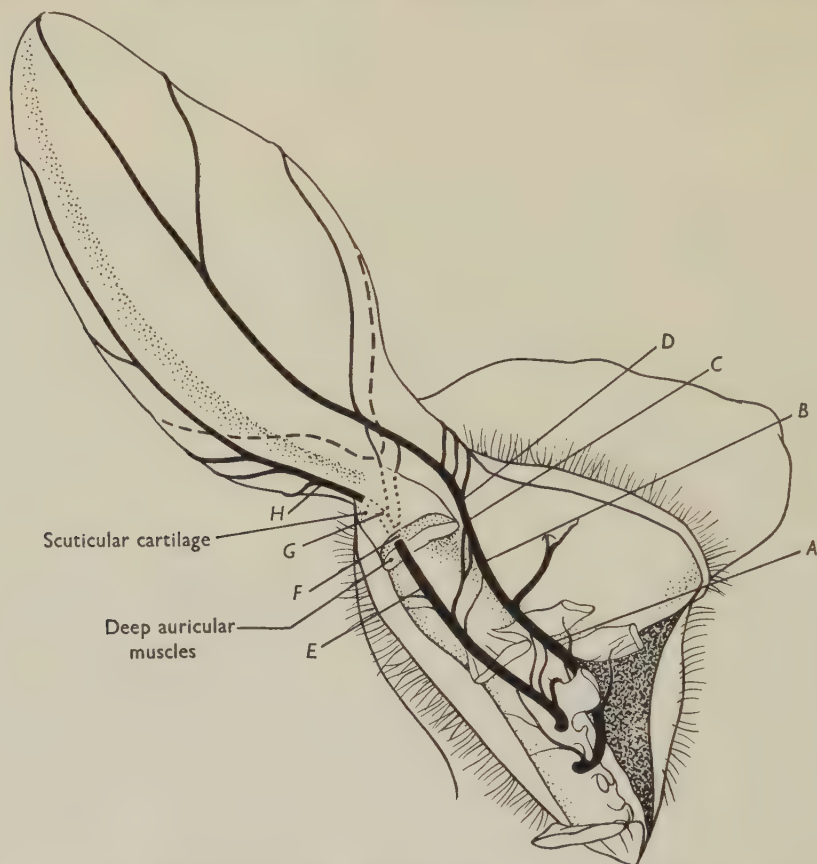


Fig. 1. Illustrates in diagrammatic form the topography of the second and third cervical spinal nerves and their relationship to the rabbit ear. Counts of the number of nerve fibres in the segment between the letters *A* to *B*, *C* to *D*, *E* to *F* and *G* to *H* were made in a number of animals (see text).

anterior primary division of the third cervical nerve, but it also receives contributions from the posterior primary division of the second cervical nerve. In fact, an interchange of fibres takes place (which varies in its detailed arrangements from rabbit to rabbit) between these two nerves in relation to the transverse processes of the first and second cervical vertebrae (Fig. 1). When the great auricular nerve is traced towards the ear it leaves the plexus and emerges from behind the posterior border of the sterno-cleidomastoid muscle, passes cranially, and crossing superficial to the insertion of the cleidomastoid, it comes to lie on the surface of the parotido-

auricularis muscle (Fig. 2). It then enters the root of the back or cranial surface of the ear by passing between the parotido-auricularis and the thin anterior group of auricular muscles. When it enters the ear it is lying directly beneath the deep fascia (i.e. between the dermis and perichondrium) lateral to the dorsal auricular artery and having the posterior auricular vein lying medially in a more superficial plane and usually overlapping it to some extent. Its further course, interconnections and main branches are seen in Fig. 1.

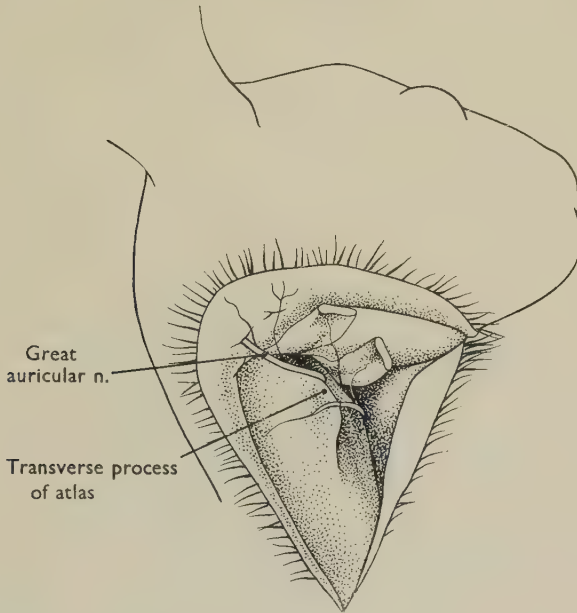


Fig. 2. Illustrates in diagrammatic form the relationship of the great auricular nerve to the transverse process of the atlas.

The lesser occipital nerve receives its main contributions from the posterior primary division of the second, but it also receives branches from the third cervical nerve (Fig. 1). The nerve emerges below the transverse process of the atlas, turns forward on the superior oblique muscle, passes through the biventer and splenius cervicis muscles to reach the dorsal surface of the skull where it lies on the lateral surface of the cervico-auricularis superficialis muscle (Fig. 3). The nerve then passes through the deep auricular muscles and scuticular cartilage and enters the dorsum of the ear under the anterior border of the fronto-scutularis muscle close to the medial margin of the auricular cartilage (Fig. 1).

The nerves entering the base of the ventral surface of the ear consist of a few fine nerve trunks from the auricular branch of the trigeminal and vagus nerves. The majority of nerves supplying the ventral surface of the ear, however, reach their destination by passing around the edge of the auricular cartilage from the dorsum. On reaching the ventral surface of the ear they interdigitate with branches from the nerves of cranial origin (see paper III of this series). Because of this, and because

of the variations in position of the cranial nerve contributions which we have encountered, no attempt has been made to indicate in simple and diagrammatic form the position and areas of skin innervated by each of these cranial nerves for such a picture would be most misleading.

(2) *Enumeration of dorsal root nerve fibres supplying the rabbit ear*

It is clear from the mode of distribution of the great auricular and lesser occipital nerves illustrated in Fig. 1 that the number of dorsal root nerve fibres supplying the ear cannot be determined simply by counting the number of nerves entering the

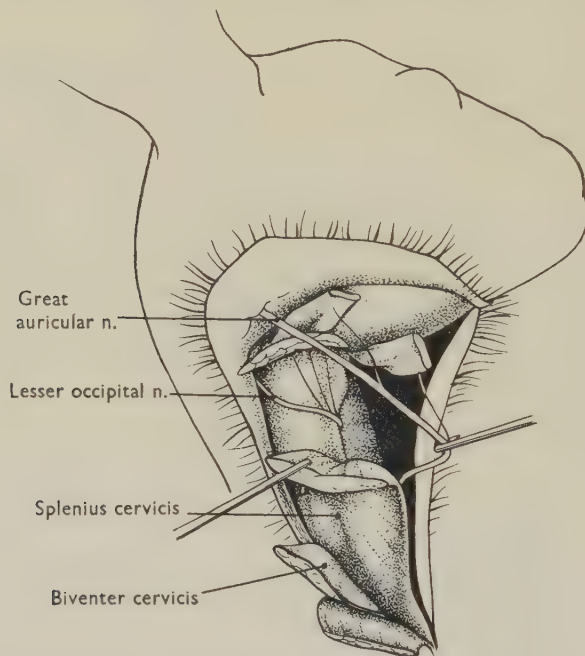


Fig. 3. Drawing from a dissection made to expose the great auricular and lesser occipital nerves in the neck.

cord at the level of the second and third cervical segments for some of the fibres come from neck muscles and from the skin of the neck and the face. Thus, before estimating the number of nerve fibres entering the base of the ear, our first objective was to determine whether multiplication of nerve fibres by branching took place to any appreciable extent between the dorsal roots and the base of the ear, for Lavarack, Sunderland and Ray (1951) and Sunderland & Lavarack (1953) have deduced that multiplication by branching probably takes place in certain human cutaneous nerves, Windle (1923), on the other hand, failed to observe multiplication of dorsal root fibres traced over long distances in dogs.

We attempted to determine whether or not multiplication was occurring by making sample fibre counts at different positions along the nerves between the dorsal roots and the base of the ear. Our observations are set out in Tables 1 and 2.



Table 1 shows that in the case of the second and third dorsal cervical nerve roots in any particular rabbit: (1) the number of axons is approximately the same on either side of the dorsal root ganglia, (2) the total number of axons available for discharging impulses into the spinal cord was around 15,000, of which rather less than two-thirds, i.e. 9500, were demonstrably myelinated, and (3) the number of

Table 1

Position	Total number of axons			Number of myelinated axons		
	$C_2$	$C_3$	$C_2 + C_3$	$C_2$	$C_3$	$C_2 + C_3$
1. Proximal to ganglion	4,850	10,586	15,436	—	—	—
2. Distal to ganglion	4,834	10,527	15,361	3,124	6,325	9,449
3. Combined motor and sensory roots	5,897	13,822	19,719	—	—	—

anterior root axons which combined to form the second and third cervical nerves were 1063 and 3295 respectively. This confirmed the observations made during the course of numerous dissections that the great auricular and lesser occipital nerves supplied relatively few muscles, being for the most part distributed to skin and, in particular, to the skin of the ear.

In view of the relatively small variations (encountered in forty dissections) in the combined size of the second and third dorsal nerve roots and in the great auricular and lesser occipital nerves which spring from them, it was considered satisfactory for our purposes to have one set of accurate counts at the positions shown in Table 1. This decision was to some extent forced on us because of the extreme difficulty of obtaining sections from the segments in question in which the individual nerve bundles and/or fibres were all accurately cut transversely. But we were able to satisfy ourselves that even in specimens in which accurate counts were not possible the number of nerve bundles present in the two roots and the approximate size of their perimeters were remarkably constant, as indeed they appeared to be when displayed by dissection.

Examination of Fig. 1 in the light of our observation that the precise point of origin, together with details of the size and course of the smaller nerve bundles leaving and acceding to the main nerve trunks, were subject to variation from animal to animal, indicates the difficulty with which we were confronted. Clearly, to obtain an unequivocal answer to the question of multiplication, it would be necessary to make counts of the nerve fibre content of the main trunks above and below each branch or accession, together with the fibre content of the branches themselves in one and the same animal. To do this is difficult, for it is impossible to obtain sections in which a sufficient number of nerve fibres are cut across transversely in regions where branching is taking place to enable accurate counting to be done.

We were thus compelled to make a number of counts in each trunk in positions free from branches or accessions in a number of different animals.

Table 2 is a sample series of counts which shows that, where it was possible to obtain lengths of the lesser occipital nerve apparently neither giving branches to, nor receiving branches from, elsewhere, the number of myelinated axons did not

increase constantly or significantly as the nerves proceeded distally. It was therefore concluded that in the case of this nerve the amount of branching taking place between the dorsal root and the base of the ear, if any, could be ignored for our purposes. It was observed, however (see Table 2) that there were always differences

Table 2. *Myelinated axons*

Rabbit A (lesser occipital nerve). Count over $\frac{1}{2}$ cm. length <i>E</i> to <i>F</i> (Fig. 1)	Rabbit B (lesser occipital nerve). Count over $\frac{1}{2}$ cm. length <i>G</i> to <i>H</i> (Fig. 1)	Rabbit C (great auricular nerve). Count over 1 cm. length <i>A</i> to <i>B</i> (Fig. 1)
3815	2738	3130
3870	2789	3890
3838	2848	4265
3637	3047	2480
3631	2708	2532
3208	2503	2711
3782	2855	
3545		
3621		
3850		

between individual counts at different levels in the stretches of the lesser occipital nerve for which at first it was difficult to account. Careful examination of the sections, however, revealed that at intervals there was a considerable interchange of axons between the fasciculi making up the nerve. Sections at positions of maximum interchange showed a number of obliquely cut axons in the fasciculi which it was not possible to enumerate with precision in addition to a number of isolated obliquely cut axons lying between fasciculi which had not been included in the count (see below).

In the case of the great auricular nerve, the sample figures in Table 2 also suggest that little multiplication by branching takes place between the dorsal root and base of the ear. In this case, however, because of numerous branches from and accessions to the nerve, the figures are of relative rather than absolute value.

These observations taken together suggested that if counts were made in positions such as between *C* and *D*, and *E* and *F* (Fig. 1) in rabbits in which the local topography made this possible we would obtain as good a measure as was possible of the number of myelinated second and third dorsal root nerve fibres which reach the skin of the ear. Such counts are set out in Table 3.

Table 3

Rabbit	Lesser occipital		Great auricular		$C_2 + C_3$	
	Total axons	Myelinated axons	Total axons	Myelinated axons	Total axons	Myelinated axons
D	3761	2855	4636	3262	8397	6117
E	3294	2437	4200	3266	7494	5703

The observations in Table 3, viewed in the light of the estimated total number of nerve fibres in the second and third cervical dorsal roots and the topographical arrangements determined by dissection, show that in young adult rabbits of the breeds chosen the total number of nerve fibres of dorsal root and autonomic origin

which enter the ear is unlikely to be much in excess of 8000 and the number of myelinated axons (presumably only of dorsal root origin) not much in excess of 6000.

Twenty counts of myelinated fibres selected at random from sections taken at various positions along the course of each of these nerves in ten animals are all of the same order as those given in the above tables, but since, as has already been pointed out, the plexiform arrangements between the lesser occipital and great auricular nerves vary from animal to animal, isolated figures from any positions other than those given in Table 3 are of relative value only, and for this reason have not been tabulated.

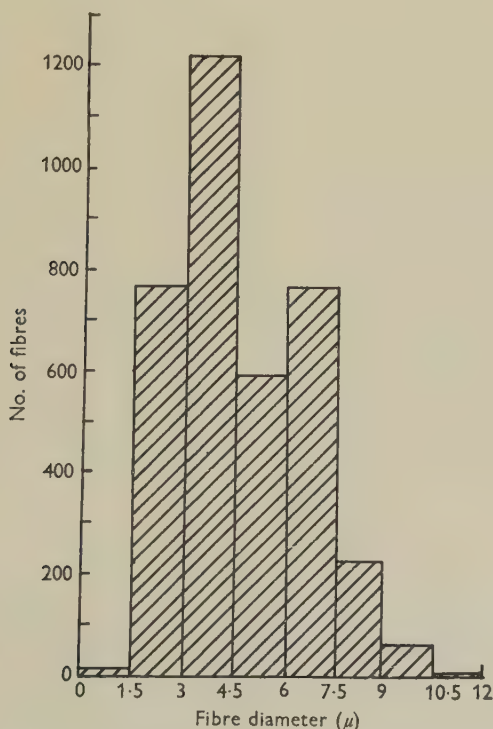


Fig. 4. Histogram made from a transverse section of the lesser occipital nerve taken from a position similar to that shown in Fig. 1 as lying between *E* and *F*.

As can be seen in Fig. 1, the only position in which it was possible to obtain a satisfactory count of the fibres contributed to the ear by the lesser occipital nerve was between *E* and *F*. An analysis of the diameters of the myelinated fibres was carried out and is given in the form of a histogram in Fig. 4. The analysis shows that the nerve in this position has a similar spectrum to that which was found by Rexed & Sourander (1949) in the cat to be typical of sensory as opposed to motor nerves. This confirmed the observations made during dissection that although the nerve is lying on muscles in this position it carries no motor fibres. The great auricular nerve, at the level at which counts were made for Table 3, is already beyond the region where it gives branches to muscles.



It has already been noted that there is considerable interchange of nerve fibres between the fasciculi forming the lesser occipital nerve; the same applies to the great auricular nerve. To illustrate this in more detail, counts over a length of 2 mm. were made of the number of myelinated axons in two fasciculi in the lesser occipital nerve lying adjacent to one another but separated from the remaining fasciculi by fat. The same thing was done in the case of three adjacent fasciculi of the great auricular nerve. The figures are set out in Table 4.

Table 4

Lesser occipital			Great auricular			
Fasc. 1	Fasc. 2	Total	Fasc. 1	Fasc. 2	Fasc. 3	Total
216	266	482	245	513	250	1008
236	265	501	254	516	214	974
215	252	467	273	553	193	1019
210	251	461				

These counts indicate (as can be readily confirmed by examining the specimens under the microscope) the measure of interchange of axons between fasciculi over relatively short distances. It is this interchange which accounts for the random variations in the counts over a length of nerve apparently free from branches or accessions for obliquely cut axons passing between bundles are not added to the total because it has been our rule to ignore anything that is not unequivocally a transverse section of a nerve fibre wholly within the fasciculus. In addition to the interchange of axons between adjacent fasciculi a continuous process of grouping and re-grouping of fasciculi is seen to be taking place as the nerves are traced either proximally or distally. This is illustrated in Fig. 5 which was made by outlining the changing fascicular pattern in serial sections of the nerves by projection at the same magnification.

### (3) *Enumeration of cranial nerve fibres supplying the skin of the rabbit ear*

The number of axons in the auricular branches of the vagus and auriculo-temporal nerves related to the dorsal and ventral surfaces of the ear were enumerated in two rabbits 2 weeks after interruption and resection of 2 mm. of the great auricular and lesser occipital nerves. The bundles counted lay in the plane between the dermis and the perichondrium in the long axis of the ear. Bundles in a more superficial plane (which never lie in the long axis of the ear in successive sections) were ignored for they belong to the cutaneous plexus which is derived by branching from dorsal root and other nerve fibres related to the ear.

Table 5

Rabbit	Total number of axons	Number of myelinated axons
F	870	640
G	720	420

The number of cranial nerve fibres entering the rabbit ear is subject to variation as can be seen in Table 5, but these differences are due in part to the difficulty of counting, for in any given position not all the bundles are cut transversely. Further

counts were therefore made of nerves entering the ventral aspect of the ear in suitably stained transverse celloidin sections through the base in unoperated specimens. In a series of five specimens none was entirely suitable for accurate counting but estimated totals averaged 300 myelinated nerve fibres.

The number of fibres in the auricular branch of the vagus on the back of the ear stained with methylene blue (which is, of course, included in the total counts in Table 5) was estimated to be  $200 \pm 30$ , of which  $150 \pm 30$  were myelinated. Counting nerve fibres in a bundle passing horizontally across the field of the microscope is not

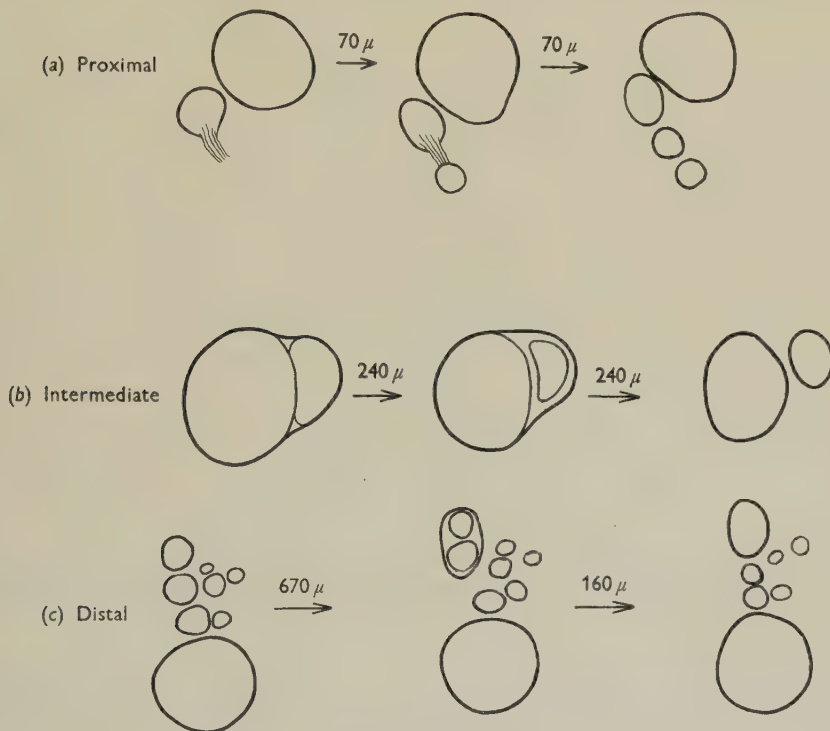


Fig. 5. Tracings of fasciculi from transverse serial sections taken from a stretch of the great auricular nerve having no branches or accessions. Diagram illustrates grouping and regrouping of fasciculi as nerve is traced distally. In (a) and (b) fasciculi are multiplying. In (c) the fasciculi are diminishing in number.

easy and the deviation considerable, for it had to be done by direct observation of the nerve at different positions along its course using an eye-piece graticule. The approximate course and area of skin over which this nerve was distributed is shown in Fig. 6. The area is, however, probably larger than it would have been when the spinal nerves were intact for the reason that nerve sprouts have probably proceeded from the vagus nerve towards the denervated area. This phenomenon has been described previously and in more detail by Grant *et al.* (1932), Weddell, Guttman & Gutman (1941), Zander & Weddell (1951), and Grant (1954).

These denervated specimens also demonstrated that some of the nerves supplying

the skin over the proximal quarter of the ear (i.e. from the base of the ear as far as the intertragic notch) were derived from branches leaving the great auricular and lesser occipital nerves above the point of section of these major nerves as they entered the ear, for the nerve bundles of the cutaneous plexus could be seen in the dermis; moreover, the animals responded to pin prick in this area. However, once again, the area covered by these branches was probably larger than it would have been before operation because of the extension of undamaged nerve fibres sprouting into the denervated zone.

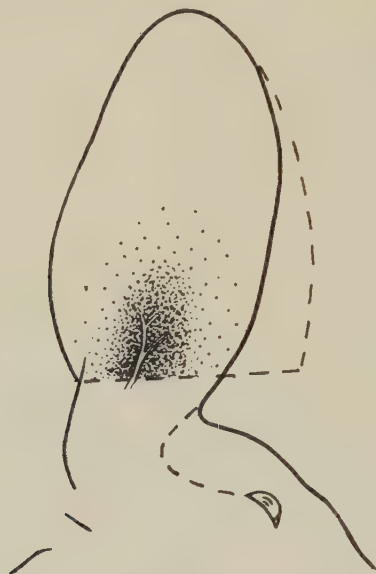


Fig. 6. Shows in diagrammatic form the approximate area of skin on the dorsum of the rabbit ear subserved by the auricular branch of the vagus; specimen taken 2 weeks after section of the great auricular and lesser occipital nerves.

It has, of course, been assumed by analogy with the spinal nerves that no multiplication by branching of axons takes place in the case of the myelinated cranial nerve contributions to the innervation of the ear. To determine the facts would be difficult if not impossible. However, it is interesting to note that in one of the operated specimens (*F* in Table 5) the total number of myelinated cranial nerves entering the ear is larger than in any of the unoperated specimens when added to the admittedly uncertain count of an operated specimen of the auricular branch of the vagus supplying the dorsum of the ear. This may have been chance or just possibly due to multiplication as the result of denervation. This point has been underlined to re-emphasize the difficulties we encountered in estimating the combined number of myelinated nerve fibres of cranial and dorsal root origin which enter the rabbit ear. Nevertheless, on the evidence at our disposal it seems not unreasonable to conclude that the average number of fibres involved is probably not in excess of 6500. It is also reasonable to conclude from the evidence we have that the average total number of non-myelinated nerve fibres entering the ear in company with myelinated nerve fibres is probably not in excess of 2500.



## DISCUSSION

Although at first sight it appeared to be a relatively straightforward task to determine the number of sensory myelinated nerve fibres supplying the skin of the rabbit ear, this did not prove to be the case. The first difficulty was due to the fact that both the major (spinal) sensory nerves carried fibres destined for neck and auricular muscles and fibres from the skin of the face and back of the neck and scalp. This ruled out the possibility of simply counting the number of sensory dorsal root nerve fibres and made it necessary to determine whether branching of the sensory nerves took place between the nerve roots and the ear. This proved difficult owing to the variable and free interchange of branches between the main spinal nerves. However, as the result of careful dissection and fibre counts in a number of selected positions (Fig. 1) it is possible to conclude that little, if any, branching of myelinated sensory nerves takes place in the main nerve trunks. It was possible to make an estimate of the number of myelinated nerves entering the root of the ear, but a second difficulty was then encountered. Owing to the free interchange of fibres between the great auricular and lesser occipital nerves at the root of the ear, it was not possible to make accurate counts in any specimen in which the plexiform arrangements did not approximate closely to that illustrated in Fig. 1. Even in specimens which were suitable in this respect and in which counts at the level of the roots could be made on the same specimen, it was not possible to estimate the number of nerve fibres in the cranial nerve components for the rabbits do not survive such extensive operations and degeneration of the spinal components is obligatory to allow a successful count. The third difficulty in obtaining accurate figures lies in the free interchange of fibres between fasciculi and the continuous re-grouping of fasciculi. This leads to a number of nerve fibres being cut across obliquely and others lying apart from fasciculi and thus giving rise to inaccurate counts. The final difficulty lies in the fact that even supposing that an exact estimate of the number of dorsal root and cranial myelinated nerve fibres *entering* the ear could be obtained in a particular rabbit, it does not follow that this is the number which *supplies* the skin of the ear for the myelinated sensory axons are widely scattered for variable distances in the cutaneous plexuses (Weddell, 1941) before they reach their destination. It is this fact, of course, which determines that there is no abrupt line demarcating the skin territory supplied by each branch from the main nerve trunk. It is therefore only possible to determine the number of myelinated nerve fibres which enter the ear at a particular level. Nevertheless, it is probably reasonable to assume that this number reflects fairly closely the actual number innervating the skin below the level of the count. Other things being equal, there will probably be as many nerves (included in the count) which have branches leaving the ear by taking a recurrent course as enter it from nerve fibres which have left the bundle to enter the skin before the point at which the count was made.

Despite the difficulties which we encountered the broad anatomical facts remained constant from animal to animal and in specimens in which accurate counts were possible it was clear that in the particular breeds we had chosen the number of fibres involved from rabbit to rabbit did not vary to any great extent. Nevertheless, it is difficult to imagine that the complexity of the patterned arrangement of the nerve

fibres in the nerve trunks supplying the ear, which make precise counts in an individual case so difficult, is fortuitous. We must therefore assume, until it is proved otherwise, that the patterned arrangement itself is of as much significance for the understanding of the mechanism of cutaneous sensibility as the number of nerve fibres involved.

#### SUMMARY

1. We have confirmed, and to some extent amplified, the observations of Grant *et al.* that myelinated sensory nerves to the rabbit ear are related via the great auricular and lesser occipital nerves to the second and third cervical spinal nerve roots.

2. We have estimated that the total number of myelinated and unmyelinated axons entering the rabbit ear in the form of nerve bundles is not likely to average more than 9000.

3. We have further estimated that the total number of myelinated axons of spinal and cranial origin which enter the ear is not likely to average more than 6500.

4. It is suggested that the patterned arrangement of the nerve fibres in the nerve trunks is of as much significance to the understanding of the mechanism of cutaneous sensibility as the number of nerve fibres involved.

This work was made possible by a grant from the Rockefeller Foundation which is gratefully acknowledged. We would also like to thank Miss Christine Court, Miss Jean Gurden, Mr R. M. Jones and Mr Frank Blackwell for their skilled technical assistance.

#### REFERENCES

- GRANT, R. T. (1954). In discussion with G. Weddell and W. Pallie on p. 142 of: 'Observations on the neurohistology of cutaneous blood vessels'. Published in *The Peripheral Circulation in Man*. A Ciba Foundation Symposium; ed. G. E. W. Wolstenholme. London: J. and A. Churchill.
- GRANT, R. T., BLAND, E. F. & CAMP, P. D. (1932). Observations on the vessels and nerves of the rabbit's ear with special reference to the reaction to cold. *Heart*, **16**, 69-102.
- LAVARACK, J. O., SUNDERLAND, S. & RAY, L. J. (1951). The branching of nerve fibres in human cutaneous nerves. *J. Comp. Neurol.* **94**, 293-311.
- LELE, P. P. (1954). Relationship between cutaneous thermal thresholds, skin temperature and cross-sectional area of the stimulus. *J. Physiol.* **126**, 191-205.
- LELE, P. P., WEDDELL, G. & WILLIAMS, C. M. (1954). Relationship between heat transfer, skin temperature and cutaneous sensibility. *J. Physiol.* **126**, 206-234.
- REXED, B. & SOURANDER, P. (1949). The calibre of central and peripheral neurites of spinal ganglion cells and variations in fibre size at different levels of dorsal roots. *J. Comp. Neurol.* **91**, 297-306.
- SINCLAIR, D. C., WEDDELL, G. & ZANDER, E. (1952). The relationship of cutaneous sensibility to neurohistology in the human pinna. *J. Anat., Lond.*, **86**, 402-411.
- SUNDERLAND, S. & LAVARACK, J. O. (1953). The branching of nerve fibres. *Acta anat.* **17**, 46-61.
- WEDDELL, G. (1941). The pattern of cutaneous innervation in relation to cutaneous sensibility. *J. Anat., Lond.*, **75**, 346-366.
- WEDDELL, G., GUTTMANN, L. & GUTMAN, E. (1941). The local extension of nerve fibres into denervated areas of skin. *J. Neurol.* **4**, 206-255.
- WEDDELL, G. & PALLIE, W. (1954). The value of 'spreading factors' in the demonstration of tissue neural elements. *Quart. J. micr. Sci.* **95**, 389-397.
- WEDDELL, G., PALLIE, W. & PALMER, E. (1954). The morphology of peripheral nerve terminations in the skin. *Quart. J. micr. Sci.* **95**, 483-501.
- WINDLE, W. F. (1923). Unmyelinated nerve fibres of the dorsal root. *J. Anat., Lond.*, **57**, 360-363.
- ZANDER, E. & WEDDELL, G. (1951). Observations on the innervation of the cornea. *J. Anat., Lond.*, **85**, 68-98.

# STUDIES ON THE INNERVATION OF SKIN

## II. THE NUMBER, SIZE AND DISTRIBUTION OF HAIRS, HAIR FOLLICLES AND ORIFICES FROM WHICH THE HAIRS EMERGE IN THE RABBIT EAR

BY G. WEDDELL AND W. PALLIE

*Department of Anatomy, University of Oxford*

### INTRODUCTION

Although many reports on the size and patterned arrangement of the hairs in various animals from different regions of the body surface are available (de Meijere, 1894, 1931; Gegenbaur, 1898; Wood-Jones, 1925; Hardy, 1947) none specifically relating to the rabbit ear could be found. The only investigation which was at all relevant was that of Bodmer-Giger (1924) who investigated the distribution of the 'guard' hairs on the back of rabbits, making estimates of the density of these hairs per cm.<sup>2</sup> of skin by the method described by Friedenthal (1912). It is this method which, with certain modifications, we used in this investigation.

Preliminary examination at once made it clear that the arrangement of the hairs in the rabbit ear is not simple, and that it would be advisable to select our material so that in addition to enumerating the hairs we should be able to extract as much additional information about them as possible. Further, the shape of the ear and the mobility of the skin between the base of the ear and scalp made it clear that to obtain from animal to animal anything approaching comparable areas of skin in which to make numerical estimations it would be advisable to use that portion of the ear distal to a line horizontal to the long axis of the ear passing through the intertragic notch at the same point in each animal, i.e. approximately the distal three-quarters rather than the whole of the ear.

### MATERIAL AND METHODS

Dutch rabbits, 3-6 months old in late summer or winter, were used since pigmentation in this breed under such conditions is confined to the margins of the holes from which the hairs emerge and to the extra-follicular portion of the hair shaft. These circumstances proved ideal for the enumeration of pigmented hair shafts and orifices from which they emerged and also of the follicles themselves for the pigment-free portion of the hairs could easily be distinguished lying within them (Pl. 1, fig. 1). Specimens were obtained during the late summer and winter months, for at other times, during periods of active follicular growth, not only are hairs lost by moulting but the size of the follicles in relation to the hairs is constantly changing. Moreover, the intra-follicular part of the hair becomes pigmented and, under such conditions, it becomes more difficult when counting to discriminate obliquely lying, extra-follicular hair shafts from those lying within follicles.

The hair on the pinna was clipped short enough to prevent the obliquely lying



hair shafts overlying and thus obscuring adjacent hair follicles when the skin was mounted between glass slides. The upper part of the ear was removed distal to a line passing horizontally to its long axis through the intertragic notch and its area determined by laying it flat on graph paper. A grid in cm.<sup>2</sup> outlined in indian ink was then stamped on to the dorsal surface. The skin was carefully stripped off the cartilage, fixed in formalin, dehydrated, cleared in xylol and, after cutting into horizontal strips with sharp scissors, mounted in 'DePex' (Gurr). Other specimens, which included skin from the ventral surface of the ear, did not have grids stamped on them, but after mounting in 'DePex' were covered with a photographic plate on which a fine graticule in numbered areas measuring  $\frac{1}{4} \times \frac{1}{3}$  cm. had been printed (Pl. 1, fig. 2). This was considered permissible since the amount of shrinkage which occurred during the preparation of specimens for examination was small. The indian ink grid was not in fact measurably different in size after processing. The mounted specimens were transilluminated and photographed at suitable magnifications for counting purposes.

#### OBSERVATIONS

A preliminary survey of this material confirmed and re-emphasized the complexity of the patterned arrangement of the hairs and made it quite clear that merely counting the number of hairs per unit area of skin and equating this with the number of myelinated nerve fibres entering it would not give us sufficient anatomical data on which to start analysing the role of hairs as sense organs. For instance, in some parts of the ear, single hair follicles give rise to two hairs. Further, the number of hairs, each derived from a separate follicle, which emerge from a single orifice in the skin, varies. In some areas the numbers lie between one and three, in other areas between one and seven. The diameters of the hair shafts and the size of the follicles from which they emerge also varies. Large follicles are usually associated with single hairs of large diameter which are the sole occupants of the orifices from which they emerge, but this is not always the case. Another striking feature is the variation in the patterned arrangement of the orifices from which the hairs emerge (Pl. 1, figs. 1, 3 and 4).

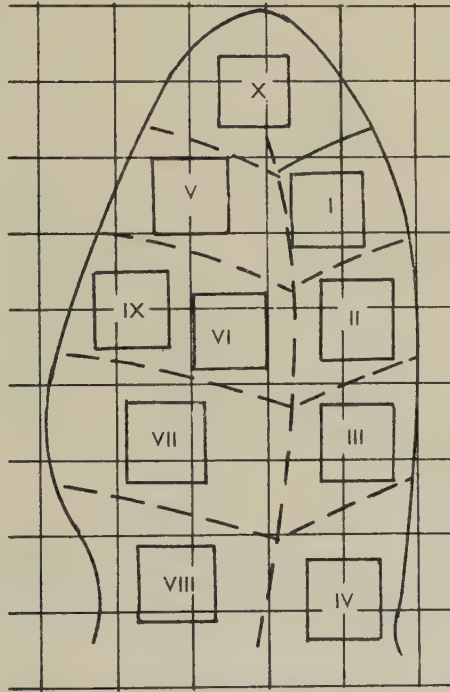
Whilst preparing specimens for examination, we became aware that certain physical properties of the hair, for instance, its length and degree of stiffness, as well as the physical properties of the skin, such as its elasticity in the region from which the hair emerges are variable and must also play a part in determining the precise reaction of individual hairs (and thus the nerves supplying the follicle) to a given mechanical stimulus. Clearly, an analysis of all these important and probably independently variable characteristics would be a very long as well as an extremely difficult task. We therefore confined ourselves in the first instance to an analysis of: (1) the number of hairs; (2) the number of hair follicles; (3) the number of skin orifices from which the hairs emerge; and (4) the approximate number of groups into which the orifices are gathered in different zones of ear skin. These are hard to enumerate accurately for, in certain regions, the hairs are more or less evenly distributed (Pl. 1, fig. 6).

Since these figures alone will give a very incomplete picture of the situation as it is in a living animal, we thought that it was both necessary and desirable to present

our observations in a form which would be most helpful in relation to our main objective. Thus we have selected a series of ears at random from those which we have examined. In each case, we have tabulated certain data and illustrated their bearing on a particular point which must be constantly borne in mind if the overall figures which have been computed are not to be used out of context.

### *Rabbit A*

The area of dorsal ear skin excised was 31 cm.<sup>2</sup>. A series of counts were made in each of the ten zones indicated in Text-fig. 1. Each zone represents 1 cm.<sup>2</sup> of skin. Pl. 1, figs 3 and 4, are photomicrographs, each at the same magnification from areas VI and II respectively. In Pl. 1, fig. 3, the hairs are seen to be arranged in



Text-fig. 1. Outline of dorsal ear skin removed from rabbit A, together with position of centimetre square areas from which counts were made.

groups consisting of three orifices; emerging from the central orifice is a single thick hair springing from a large follicle; it is flanked by two orifices, from each of which emerge three thinner hairs, springing from small closely associated follicles. In Pl. 1, fig. 4, the picture is quite different. The orifices are closer to one another and their grouping is much less regular. In one position, as many as seven fine hairs can be seen emerging from a single orifice, although at least one example of a single large hair associated with a single orifice can be seen. In this and in other areas in which the hairs are arranged in a similar way, two hair shafts sometimes emerge from a

single orifice associated with a single hair follicle. Pl. 1, figs. 1, 3 and 4, has been chosen to indicate the kind of variation which is seen, and the difficulty there is in expressing this quantitatively, for as will be demonstrated shortly, the change in pattern is a continuously variable one between the extremes which we have illustrated. Nevertheless, figures can be given which represent the range of variation encountered, and Table 1 summarizes the observations made in the case of this particular specimen.

Table 1

Area (Each 1 cm. <sup>2</sup> )	Groups of orifices	Orifices	Hairs per orifice		Follicles per orifice	
			Range	Average	Range	Average
1	218	622	1-4	3	1-4	2.2
2	200	607	1-7	4.2	1-6	3.7
3	155	490	1-5	3.6	1-4	3.3
4	192	591	—	—	—	—
5	228	692	1-6	3.3	1-4	3.0
6	186	523	1-5	3.3	1-5	3.1
7	165	479	—	—	—	—
8	172	454	—	—	—	—
9	—	—	—	—	—	—
10	167	446	1-4	2.1	1-4	2.0
Average	186	533	—	3.3	—	3.1

These figures can be used to estimate the average number of (1) hair shafts, (2) hair follicles, (3) orifices from which the hairs emerge, and (4) groups into which the orifices from which the hairs emerge are gathered in the dorsum of the distal three-quarters of the rabbit ear. Thus in 31 cm.<sup>2</sup> of skin there will be approximately 54,526 hair shafts, 51,221 hair follicles, 16,532 orifices and 5766 groups of orifices from which the hairs emerge. It is, however, clear that it would be most unwise to draw any conclusions from equating any or all of the average figures thus obtained with the number of myelinated nerve fibres which enter the ear.

#### *Rabbit F*

In order to check the accuracy of our sampling method, and in an attempt to illustrate the difficulties with which we were confronted from another point of view, we removed an ear from a 3-month-old rabbit which was thin enough to be mounted in strips as a single preparation and covered with a fine graticule consisting of numbered areas measuring  $\frac{1}{3} \times \frac{1}{4}$  cm. The preparation was photographed square by square and the number of hairs in each square was recorded. It was impossible to make accurate counts along the edge of the ear in some regions owing to the obliquity, thickness and overlapping of the hair stumps. In such instances, numbers proportional to those obtained in the adjacent or nearest squares were added to the total. The area of ear skin involved was 30 cm.<sup>2</sup> and the total number of hairs proved to be 53,137, a figure which is very close to the one which we found in rabbit A and which was arrived at by the sampling method, the reliability of which is thus confirmed.

Photographs used for making the counts in rabbit F, all at the same magnification, were mounted in their correct sequence and each hair shaft, save for some of those at the edge of the ear, was accurately outlined in indian ink. The photographic background was then bleached away and the result is illustrated in Text-fig. 2. This



shows the relative density and thickness of the hair stumps emerging from each orifice and the pattern in which they are arranged but the magnification is too low for individual hairs to be visualized.

This method demonstrates clearly that grand totals give no idea of the complexities of the actual patterned arrangement of the hairs and hair follicles; rather, they iron out irregularities and are thus liable to give a totally false impression of the real situation if used out of context.



Text-fig. 2. Patterned arrangement and total thickness of hair stumps emerging from orifices in dorsal ear skin, rabbit F. Figure made by outlining stumps in indian ink on serially mounted photomicrographs and subsequently bleaching away background. Magnification too low to distinguish individual hairs emerging from a single orifice.

### *Rabbit K*

It is possible to break down the pattern shown in Text-fig. 2 into some of the individual patterns of which it is composed. Photographs of areas measuring  $\frac{1}{3} \times \frac{1}{4}$  cm. were taken in the positions indicated in Text-fig. 3. Counts were made of the total number of orifices in each area from which the hairs emerged as well as the number of distinct groups into which the orifices were segregated. The numbers so obtained have been inserted within the circles outlining the areas sampled in Text-fig. 3. These numbers (together with less extensive samples, which were taken in order to complete the picture, from areas which had not been as thoroughly covered) were used to construct Text-figs. 4 and 5 which show in graphic form the relative density of the orifices and hairs respectively over the dorsum of the distal

three-quarters of the rabbit ear. It will be seen that the patterned arrangement in Text-fig. 4 is different from that in Text-fig. 5.

Calculations based on figures obtained by sampling in other specimens gave results which were of the same order as those in the ears already described. Figures obtained in the case of five animals selected at random are given in Table 2. The figures



Text-fig. 3. Outline of dorsal ear skin removed from rabbit K. The circles indicate the areas from which accurate counts were made. The numbers within the circles are the average figures for orifices over groups of orifices respectively.

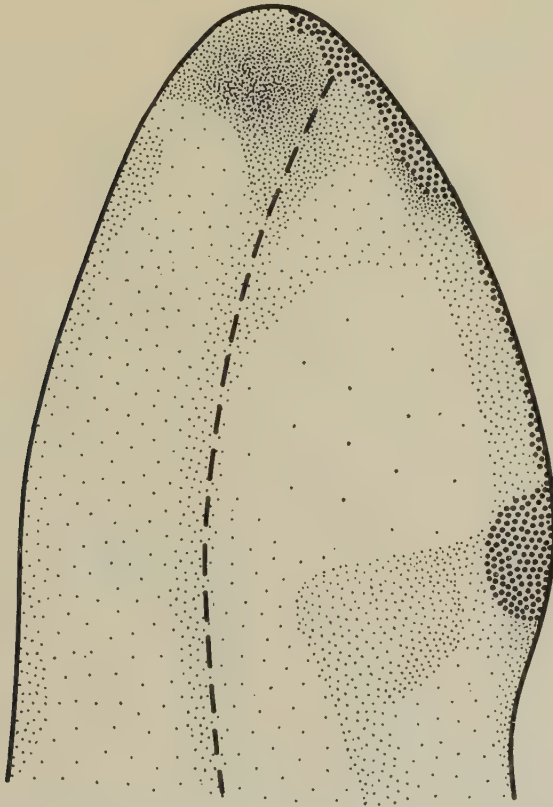
Table 2

Rabbit	Hairs	Orifices
A	54,526	16,532
F	53,137	—
P	55,220	18,408
Q	—	19,434
R	53,315	17,884

obtained are remarkably consistent and appear to bear no relation to the size of the ear, although to have put this on a firm statistical basis in the adult would have meant an inordinate amount of work for the size of the ears in the adult animals we used did not vary very greatly. For this reason, we took a specimen from a 3-weeks old rabbit.

*Rabbit Q*

In this instance, it was found possible to mount the piece of ear excised between glass slides without cutting it into strips, the covering slide bearing a grid of numbered areas measuring  $\frac{1}{3} \times \frac{1}{4}$  cm. (Pl. 1, fig. 2). Each area was photographed and a count made of the orifices from which the hairs emerged. It was decided to count orifices rather than hairs or follicles because the figures for the orifices were found to



Text-fig. 4. Illustrates graphically the relative density of orifices in rabbit K. This figure was constructed from Text-fig. 3, together with additional sample counts in zones not covered in Text-fig. 3.

be the most consistent and reliable of the counts from animal to animal. This result might have been expected for the optical plane of the orifices was the one brought into sharp focus in each photograph so as to obtain pictures in which the depth of focus was sufficient to allow both shafts and follicles to be counted at the same time. The total number of orifices proved to be 19,434 in an area of 14 cm.<sup>2</sup>. This figure is of the same order as that found in the case of adult rabbits. It is, in fact, slightly larger than the average figure for the ears used to illustrate other points in this paper and can be explained on the basis that rather more than the distal three-



quarters of the ear was removed as the result of an irregular incision through the skin (Pl. 1, fig. 2).

This particular observation is not at variance with those reported by previous observers that the number of hair follicles in a given area does not alter appreciably after birth (Oshima, 1907; Wolbach, 1951). However, it would suggest that tactile acuity would alter during the course of growth if the arrangement of the sense organs was such that each hair was simply and exclusively related to a single dorsal root nerve fibre.



Text-fig. 5. Illustrates graphically the relative density of groups of orifices in rabbit K. Also constructed from Text-fig. 3, together with additional sample counts in zones not covered in Text-fig. 3.

#### *Rabbit F*

In the case of adult ears, we thought it would be worth while, from the point of view of estimating possible differences in tactile acuity, to make some sort of estimate of the distances between hair groups. From the data which we had in our possession it was possible to calculate the distances between the geometrical centres of the hair groups, assuming that they were evenly distributed within each area measuring  $\frac{1}{3} \times \frac{1}{4}$  cm. (which is, of course, not strictly true). Calculations were based on both the squared and equilateral lattice formulae (see Appendix) and the results of each calculation are shown graphically in Text-fig. 6. From this figure, it can be seen that the distance between the geometric centres of groups varies from place

to place between the limits of 0.51–0.85 mm. It has further been ascertained by direct measurement from photomicrographs that the average diameter of a circle delineating the area occupied by a single group is 0.35 mm. From this it may be calculated that the average linear length of skin free from hairs is 0.2–0.5 mm. This means that the size of a stimulus object must certainly be less than 0.5 mm. in cross-sectional diameter if it is to be used to strike a single group of hairs. These calculations are based on measurements made after the hair had been clipped. When the



Text-fig. 6. Outline of dorsal ear skin removed from rabbit F. The numbers in the circles represent the average distance separating follicle groups in the positions indicated, calculated on the basis of the squared lattice arrangement. The numbers in the squares represent the distance in the position indicated, calculated on the basis of the equilateral lattice arrangement.

ear is in its natural state the hairs overlap one another extensively so that it would become even more difficult to strike a single group of hairs by any form of contact as opposed to nociceptive stimulus object.

#### *Rabbits, D, G and H*

The hairs over the anterior aspect of the ear are not so numerous. They are, however, arranged in much the same way as over the dorsum, that is, in a continuously variable pattern. The orifices from which the hairs emerge are not so

obviously in groups which can be segregated by casual inspection and it is more common for each orifice to have but a single hair emerging from it (Pl. 1, figs. 5, 6). The range, however, remains at between one and seven hairs. As in the case of the back of the ear, a single follicle is occasionally associated with two hairs. The size of the hairs and the follicles from which they arise varies from region to region, the range being much the same as in skin from the dorsum but the actual number of large hairs and large follicles is considerably less. In view of the results we had obtained in relation to the dorsal skin, we decided to estimate, by sampling only, the number of hairs, the number of orifices from which the hairs emerged, together with the number of groups into which the orifices were gathered in the front of the distal three-quarters of the ear. This gave us the following mean figures: hairs 22,336, orifices 15,040, groups 5632. These figures are of considerable interest for they show that there are approximately half the number of hairs and follicles on the front of the ear, but that there are approximately the same number of orifices and groups of orifices from which the hairs emerge.

#### *Rabbit T*

Finally, sample counts were made of the number of hairs in representative areas of skin from the back and front of the proximal quarter of the ear. Counts in the ten areas measuring  $\frac{1}{3} \times \frac{1}{4}$  cm. examined suggested that both the patterned arrangement and the number of hairs are of the same order as those in the distal three-quarters of the ear. In view of the difficulty of assessing the precise area of skin comprising the ear (for reasons to which we have already referred), and in view of the detailed observations which we have already made in relation to the distal three-quarters of the ear, we considered that a more detailed analysis of the number and patterned arrangement of the hairs in the proximal quarter of the ear was unlikely to be profitable in relation to our objective. We have, therefore, assumed that the proximal quarter of the ear has the same proportional number of hairs as the distal three-quarters. On this assumption, it is reasonable to conclude that the average number of hairs in the Dutch rabbit ear during periods when active follicular growth is not in progress is in the neighbourhood of 100,000, the number of orifices from which hairs emerge in the neighbourhood of 40,000 and the number of groups into which the orifices are clustered in the neighbourhood of 13,000. The last of these figures is of particular interest and possibly of some significance for it is about twice that of the number of myelinated nerve fibres entering the ear.

#### DISCUSSION

Our original intention was to equate the number of myelinated sensory nerve fibres entering the ear with the number of hairs in the ear, hoping that this would give us a figure that would enable us to retain or reject certain conceptions regarding the mechanism underlying cutaneous sensibility.

This, it is now clear, is not possible for the hairs are so arranged that from the morphological point of view a single hair cannot be regarded as representing a sensory unit. As many as seven separate hairs derived from as many closely related hair follicles may emerge from a single small orifice in the skin. Even if it were



possible to stimulate one such hair in isolation by artificial means, this clearly would not occur in the course of everyday life. In view of this, it seemed logical to consider that the number of orifices from which the hairs emerged might be directly related to the number of sensory units present. Thus it might be more reasonable to equate the number of myelinated nerve fibres entering the ear with the number of orifices from which hairs emerged, rather than with the total number of hairs present. On the other hand, it might be argued on the grounds of size *vis-à-vis* naturally occurring stimuli that it was even more reasonable to equate the number of nerve fibres with the number of groups or clusters of orifices. At first sight, this notion seemed attractive in view of the fact that the number of myelinated nerve fibres entering the ear was about half the number of groups into which the orifices were gathered. However, such a relationship is not as simple as it at first appears. Although the number of groups of orifices from which the hairs emerge is of the same order in skin from both the front and back of the ear, the number of nerve fibres supplying the front of the ear is less than the number supplying the back of the ear, probably in proportion to the number of hairs (see paper III). Moreover, in certain regions of skin the hairs are more or less evenly distributed so that it is very difficult to enumerate groups with any degree of accuracy. These factors tend to discount the close correlation which generalization at first suggested.

Over and above this is the indisputable fact that the density and arrangement of the unclipped hairs is such that even the smallest contact, as opposed to nociceptive stimulus, is certain in most areas to involve a number of hairs and through them to come into relationship with a number of hair follicle groups. In other words, the hairs are so arranged that the chance of a single hair follicle or even a single group of hair follicles being stimulated by any commonly occurring natural contact stimulus is negligible. It must be remembered that this conclusion has been arrived at on the assumption that each hair when stimulated moves freely in the skin and in so doing only stimulates the nerves ending in the follicle from which it springs. Stimulation of clipped hairs in a living animal under the binocular microscope suggests that this assumption is unlikely to be correct except under experimental conditions, and that even the movement of a single relatively isolated hair more than a very short distance probably displaces a number of follicles in its immediate neighbourhood. This, and other factors to which we have referred but not inquired into, serve to reinforce the view that the smallest naturally occurring contact stimulus is likely to involve a number of hair follicle groups.

In addition to this, it has been noted that the hairs are so arranged that their relationship to one another varies from place to place. It varies independently of the number of hair follicles to which the hairs are related, the orifices from which they emerge and the manner in which these orifices are grouped in relation to one another. The arrangement is not a random one but is patterned in a complicated manner which, although it is generally similar from rabbit to rabbit, is so arranged that a given hair is uniquely related to the hairs which surround it.

These observations make it quite clear that our original intention of equating the number of sensory myelinated nerve fibres entering the ear with the number of hairs in the ear will not give us a figure which is of unqualified significance in relation to the sensory information available to the animal when the hairs are stimulated.

Nevertheless, we are justified in concluding that there is an average number of 100,000 hairs in the rabbit ear of the breed we have examined and that there are at the most 6500 myelinated nerve fibres available to innervate the follicles from which they arise. The manner in which the sensory myelinated nerve fibres are related to the hair follicles, as well as to each other, in the skin, the spinal nerve trunks and spinal nerve roots will be the subject of the final paper in this series.

#### SUMMARY

The authors have established the following points:

1. The average number of hairs in the ear of Dutch rabbits when no active follicular growth is taking place is of the order of 100,000. The number of hair follicles is approximately 4 % less than this.

2. The number of orifices from which hairs emerge is of the order of 40,000 and the number of groups into which orifices are gathered is of the order of 13,000.

3. The number of hairs in the skin over the front of the ear is about half that in the skin over the back of the ear; the number of orifices and groups of orifices, however, is about the same.

4. The number of hairs is not related to the size of the ear, being of the same order in skin from the back of the ear of a 3-week-old rabbit (14 cm.<sup>2</sup> skin) and an average adult rabbit (31 cm.<sup>2</sup> skin).

5. The average area occupied by a group of orifices is 0.25 mm.<sup>2</sup> and the average linear distance between hair stumps is 0.35 mm.

6. The hairs are so arranged that the chance of a single hair follicle or group of follicles being stimulated by any commonly occurring natural contact stimulus is negligible. Further, the hairs are arranged in a complicated non-recurrent pattern, which is similar from rabbit to rabbit, but which makes the relationship of a given hair to those surrounding it unique.

7. Their observations make it clear that equation of the number of sensory myelinated nerve fibres entering the ear with the number of hairs in the ear will not give us a figure which is of unqualified significance in relation to the sensory information available to the animal on stimulation of the hairs.

This work was made possible by a grant from the Rockefeller Foundation which is gratefully acknowledged. We would also like to thank Miss Christine Court, Miss Jean Gurden, Mr R. M. Jones and Mr Frank Blackwell for their skilled technical assistance.

#### APPENDIX

To obtain the figures quoted in the text we formulated the following proposition:

Given  $n$  groups in an area  $A$  cm.<sup>2</sup>, what is the mean separation of the groups, i.e. what is the distance between any two groups if the  $n$  groups are dispersed in the area  $A$  such that the distance between any two adjacent groups is constant?

(a) For constant separation of the groups, these can be considered to be disposed at the intersections of a *squared lattice* of element  $d$  cm. in which each group occupies  $d^2$  cm.<sup>2</sup>.

Therefore

$$A/n = d^2, \quad \text{i.e. } d = \sqrt{A/n}.$$

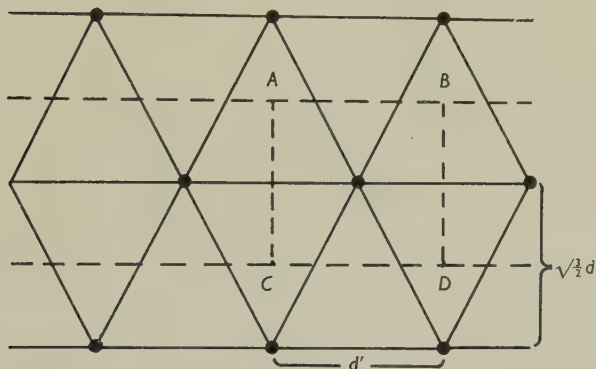
(b) Constant separation is also obtained if the groups are disposed at the intersections of an equilateral lattice (Text-fig. 7). In this case, each group occupies an area  $(d'\sqrt{\frac{3}{2}}d')$ , where  $AB=CD=d'$  and  $AC=BD=\sqrt{\frac{3}{2}}d'$ ; i.e.

$$An/\sqrt{\frac{3}{2}}d' = 0.866 d'^2,$$

i.e.

$$d'^2 = 1.55 A/n,$$

$$d' = 1.075\sqrt{(A/n)}.$$



Text-fig. 7. Illustrates diagrammatically the disposition of hair follicle groups at the intersections of an equilateral lattice.

#### REFERENCES

- BODMER-GIGER, H. (1924). Äussere Unterscheidungsmerkmale, insbesondere solche des Haarkleides der schweizerischen Feld- und Alpenhasen. *Z. indukt. Abstamm.- u. VererbLehre*, **35**, 1-105.
- FRIEDENTHAL, H. (1912). Zur Technik der Untersuchung des Haarkleides und der Haare der Säugetiere. *Z. Morph. Anthr.* **14**, 441-452.
- GEGENBAUR, C. (1898). *Vergleichende Anatomie der Wirbelthiere*, pp. 141-151. Leipzig: W. Engelmann.
- HARDY, M. H. (1947). Group arrangement of hair follicles in mammalian skin. *Proc. roy. Soc. Qd*, **58**, 125-148.
- MEIJERE, J. C. H. DE (1894). Ueber die Haare der Säugethiere besonders über ihre Anordnung. *Morph. Jb.* **21**, 312-425.
- MEIJERE, J. C. G. DE (1931). In *Handbuch der vergleichenden Anatomie der Wirbeltiere*, Vol. 1, 585-632. Vienna: Urban and Schwarzenberg.
- OSHIMA, T. (1907). Die Beziehungen des Wollhaares des Neugeborenen zu den Haaren des Erwachsenen. *Pflüg. Arch. ges. Physiol.* **117**, 341-344.
- WOLBACH, S. B. (1951). The hair cycle of the mouse and its importance in the study of sequences of experimental carcinogenesis. *Ann. N.Y. Acad. Sci.* **53**, 517-536.
- WOOD-JONES, F. (1925). On the causation of certain hair tracts. *J. Anat., Lond.*, **59**, 72-79.

#### EXPLANATION OF PLATE

##### PLATE 1

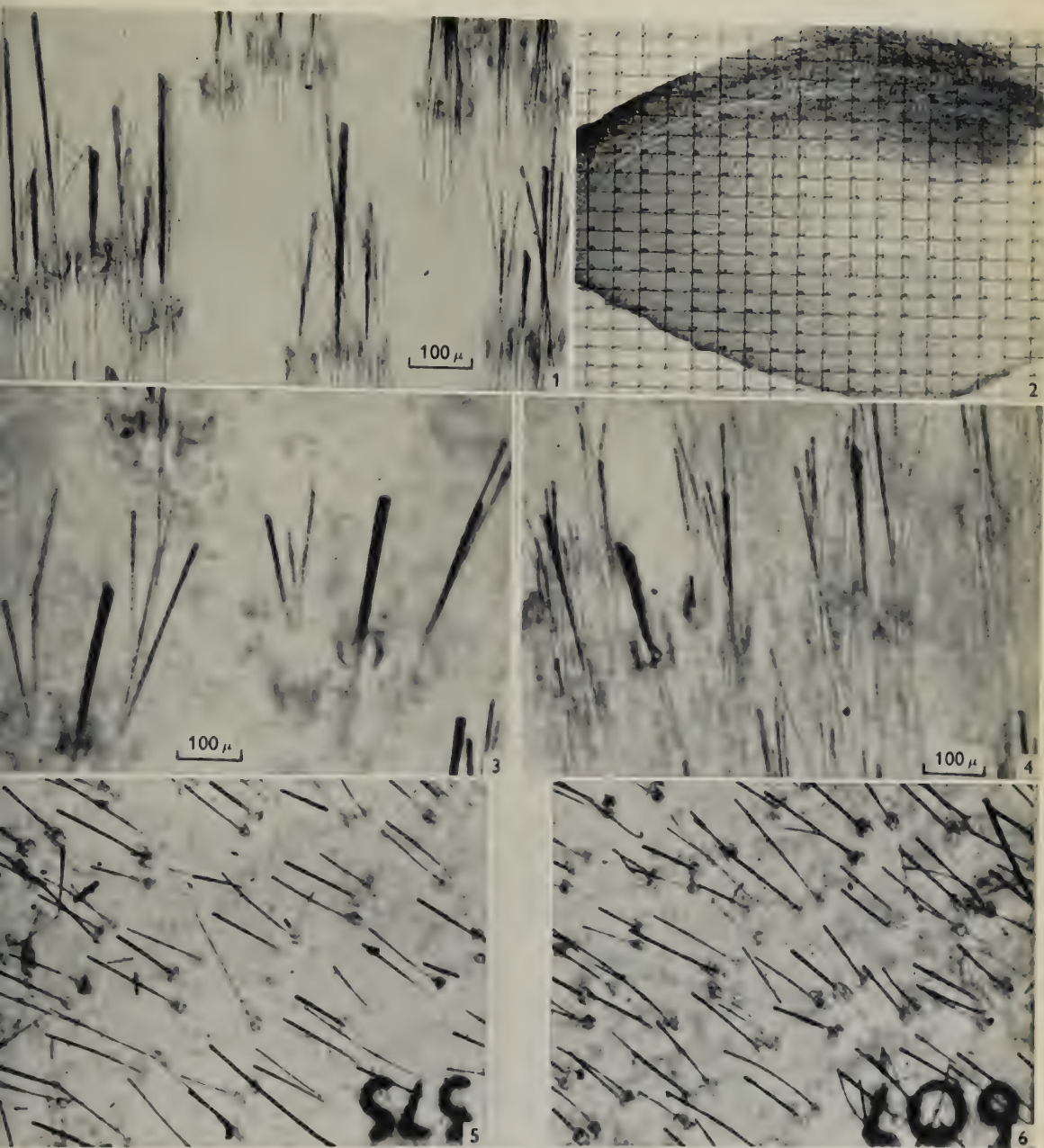
Figs. 1-5. Photomicrographs of dorsal ear skin from Dutch rabbit, 3 months old in October, showing pigmented hair shafts emerging from orifices in which surrounding skin is also pigmented. The hair shafts lying within the follicle are free from pigment.

Fig. 1. Photomicrograph of dorsal ear skin, area IV, Text-fig. 1. Compare figs. 3-6.

Fig. 2. Distal three-quarters of an ear from a Dutch rabbit, 3 weeks old, mounted as a whole specimen and covered by a printed graticule outlining areas  $\frac{1}{2} \times \frac{1}{2}$  cm.



- Fig. 3. Photomicrograph of dorsal ear skin, area vi, Text-fig. 1. Note variations in arrangements. Compare figs. 1 and 4-6.
- Fig. 4. Photomicrograph of dorsal ear skin, area ii, Text-fig. 1. Note variations in arrangements. Compare figs. 1, 3 and 5.
- Fig. 5. Photomicrograph of ventral ear skin. Note that: (1) figs. 5 and 6 are neighbouring  $\frac{1}{8} \times \frac{1}{4}$  cm. areas taken from the centre of the ear, fig. 6 lying immediately distal to fig. 5; (2) the rapid variation in pattern and density over a short distance. The borders of the print outline an area  $\frac{1}{8} \times \frac{1}{4}$  cm. Compare figs. 1, 3, 4 and 6.
- Fig. 6. Photomicrograph of ventral ear skin. Compare figs. 1 and 3-5.







# A NOTE ON THE INNERVATION OF HUMAN DENTINE

BY RALPH COCKER AND JEAN M. HATTON

*King's College Hospital Dental School (University of London)*

Clinical experience indicates that dentine is a highly sensitive structure, but the question whether nerve fibres are present in calcified dentine has been a matter of controversy for many years. Wellings (1940) reviewed some of the evidence, especially the claims made by Mummery (1924), and concluded that nerve fibres do in fact enter calcified dentine, although he fully recognized the difficulty of distinguishing nerve from connective tissue fibres with the silver impregnation techniques commonly used. Bradlaw (1939) also claimed to have traced undoubted nerve fibres into the dentine although the figures which he published in support of this claim show fibres only in the predentine or odontogenetic zone. His fig. 6 is a possible exception to this statement, and is said to show a nerve fibre entering a dentinal tubule. The section of which the figure is a photograph is, however, cut transversely to the direction of the tubules. The fibre ends apparently in the cavity of a tubule, but whether it passed along it into the calcified dentine is impossible to say without evidence from sections cut parallel to the direction of the tubule.

More recently, Powers (1952) found an axon in a molar tooth of a rat which could be traced from the pulp through the predentine and into the calcified dentine in serial sections. She used the Romanes (1950) and Ungewitter (1951) techniques of silver impregnation. She also observed numerous fibres branching and forming loops in the predentine where, of course, they have frequently been seen in human teeth.

The present position therefore seems to be that nerve fibres in the pulp and in the predentine have been demonstrated as convincingly as is possible with histological techniques, and their presence is generally accepted. It is also believed by several authorities that nerve fibres extend into the calcified dentine, possibly along the dentinal tubules, though, apart from Powers's observation in the rat, the histological evidence produced is not as satisfactory as it could be.

A difficulty arises in the use of the words 'dentine' and 'predentine'. From the literature it would seem that some authors use 'dentine' to mean the whole mass of dentine from the layer of odontoblasts in the pulp to the outer surface. This prevents any deductions being drawn as to how far the axons, which are described, penetrate into the dentine. From the published photomicrographs (except for Powers), they appear to penetrate only into the odontogenetic zone (predentine) but no farther. Lewinsky & Stewart (1938) state that the only author who has claimed to have described and photographed nerve fibres in the 'ossified' dentine is Tojoda (1934), but he was not able to show the continuity of these fibres with those of the pulp as his specimens consisted only of portions of the dentine without connexion with the pulp. We feel that unless the continuity of nerve fibres can be traced from the pulp through the odontogenetic zone and into the calcified dentine, the histological proof of the innervation of this tissue is not yet achieved.

More recently, Powers (1952) has satisfied the criterion stated as the axon she shows in the rat molar passes from the pulp across the predentine and penetrates into the calcified dentine. This she says was inked-in in the photograph to bring its whole course into view. In the circumstances it seemed desirable to publish the following observations which were made in human teeth and illustrated by untouched microphotographs.

#### MATERIAL AND TECHNIQUE

Freshly extracted and non-carious teeth were fixed for at least 3 days in 10% neutral formol saline. The mesial and distal surfaces were then ground under water until only a thin slice (but containing the whole pulp) remained. The object of this was to reduce the amount of material requiring decalcification, so decreasing the time required to complete the process.

Decalcification was carried out in a constant drip bath with 1% formic acid in saturated calcium phosphate (Brain, 1949, 1953) and, according to the size of the tooth, required 3-8 weeks for completion. Stronger concentrations of formic acid or any of the commoner decalcifying agents (e.g. nitric acid, trichloroacetic acid) were found to be unsatisfactory since they appeared to inhibit the impregnation of the finer nerve fibres in subsequent operations.

After decalcification the specimens were washed for 24 hr. in running water, dehydrated, cleared and double-embedded in celloidin and paraffin wax. Sections were cut at  $8\mu$ , and treated with a silver impregnation technique, described by Ungewitter (1951), in which urea is used to accelerate the procedure and improve the specificity of the impregnation. It was found that the addition of 3-4 drops of pure pyridine to the urea-silver nitrate solution used for impregnation improved the sharpness and general clarity of the final result, but otherwise the method was carried out as described by Ungewitter.

#### OBSERVATIONS

Sections showed the usual plexiform arrangement of nerve fibres in the pulp; varicosities on some of the larger fibres were quite common (Pl. 1, fig. 1), and seemed very similar in appearance to those interpreted by Mohiuddin (1950) as evidence of degeneration. Their frequent appearance in otherwise normal pulp tissue is against this interpretation. Numerous fibres enter the predentine (Pl. 1, fig. 2), where some run parallel with the tubules and Tomes's fibres; branching is common in this situation; some fibres are beaded, and some loop back into the pulp. All these appearances have been reported on many previous occasions, e.g. by Bradlaw (1939) and Powers (1952).

In some sections fibres could be traced from the pulp, through the predentine and into the calcified dentine, reaching in a few cases as far as half-way through the thickness of the calcified dentine. These long fibres run parallel to the direction of the tubules, but it was not possible to determine whether they were actually within the tubules or between them in the matrix. In many sections where the predentine contained numerous fine fibres, some of these could be traced into the calcified dentine where they were very soon lost. A few only of the long fibres such as those

illustrated (Pl. I, fig. 3, 4) were found; they may of course be present in much larger numbers, since only those which lie for some distance in the plane of section can be identified with certainty.

#### DISCUSSION AND CONCLUSIONS

It is well recognized that the demonstration of nerve fibres by silver impregnation methods is open to misinterpretation, the most common cause for which is the mistaking of connective tissue fibres for nerve axons. In the present specimens it can be said that the axons seen in the calcified dentine and predentine all show the sharp almost black impregnation which is characteristic of the fibres in the pulp where their identification as nerves would not be seriously questioned. Moreover, they could be traced through the predentine, between the odontoblasts, into continuity with the fibres in the pulp, which showed a similar kind of impregnation, differing markedly from the golden-brown coloration of the connective tissue fibres. The latter also pursue a far more wavy course than the axons and never show the varicosities which, whatever their significance, are a characteristic feature of many of the nerves. It is probable that final proof that these fibres in the calcified dentine are nerves could be obtained only by experimental methods, e.g. by the observation of degeneration phenomena after cutting the nerve trunks to the teeth. These methods are not practicable with human material. In the absence of this 'final proof' it is justifiable to conclude that fibres pass for a considerable distance into human calcified dentine which, so far as histological methods are capable of showing, are in fact nerve fibres.

#### SUMMARY

1. The combined use of slow decalcification and a modified silver nitrate staining technique has confirmed the course of nerve axons in the pulp and predentine of human teeth.

2. In addition the method has shown for the first time a small number of axons penetrating deeply into the calcified dentine of human teeth.

The authors would like to express their grateful thanks to George Harwood, A.I.M.L.T., of the Department of Morbid Anatomy, King's College Hospital Medical School, for his very skilful photomicrography.

#### REFERENCES

- BRADLAW, R. (1939). The histology and histopathology of the dental innervation. *Proc. R. Soc. Med.* **32**, 1040-1053.
- BRAIN, E. B. (1949). A method of preparing decalcified serial sections in paraffin wax of human enamel and dentine *in situ*. *Brit. dent. J.* **87**, 199-205.
- BRAIN, E. B. (1953). Personal communication.
- LEWINSKY, W., & STEWART, D. (1938). An account of our present knowledge of the innervation of the teeth and their related tissues. *Brit. dent. J.* **65**, 687-700.
- MOHIUDDIN, A. (1950). The fate of the nerves of the deciduous teeth. *J. Anat., Lond.*, **84**, 319-323.
- MUMMERY, H. J. (1924). *Microscopic and General Anatomy of the Teeth*, 2nd ed. Oxford.
- POWERS, M. M. (1952). The staining of nerve fibres in teeth. *J. dent. Res.* **31**, 383-392.
- ROMANES, G. J. (1950). The staining of nerve fibres in paraffin sections with silver. *J. Anat., Lond.*, **84**, 104-115.

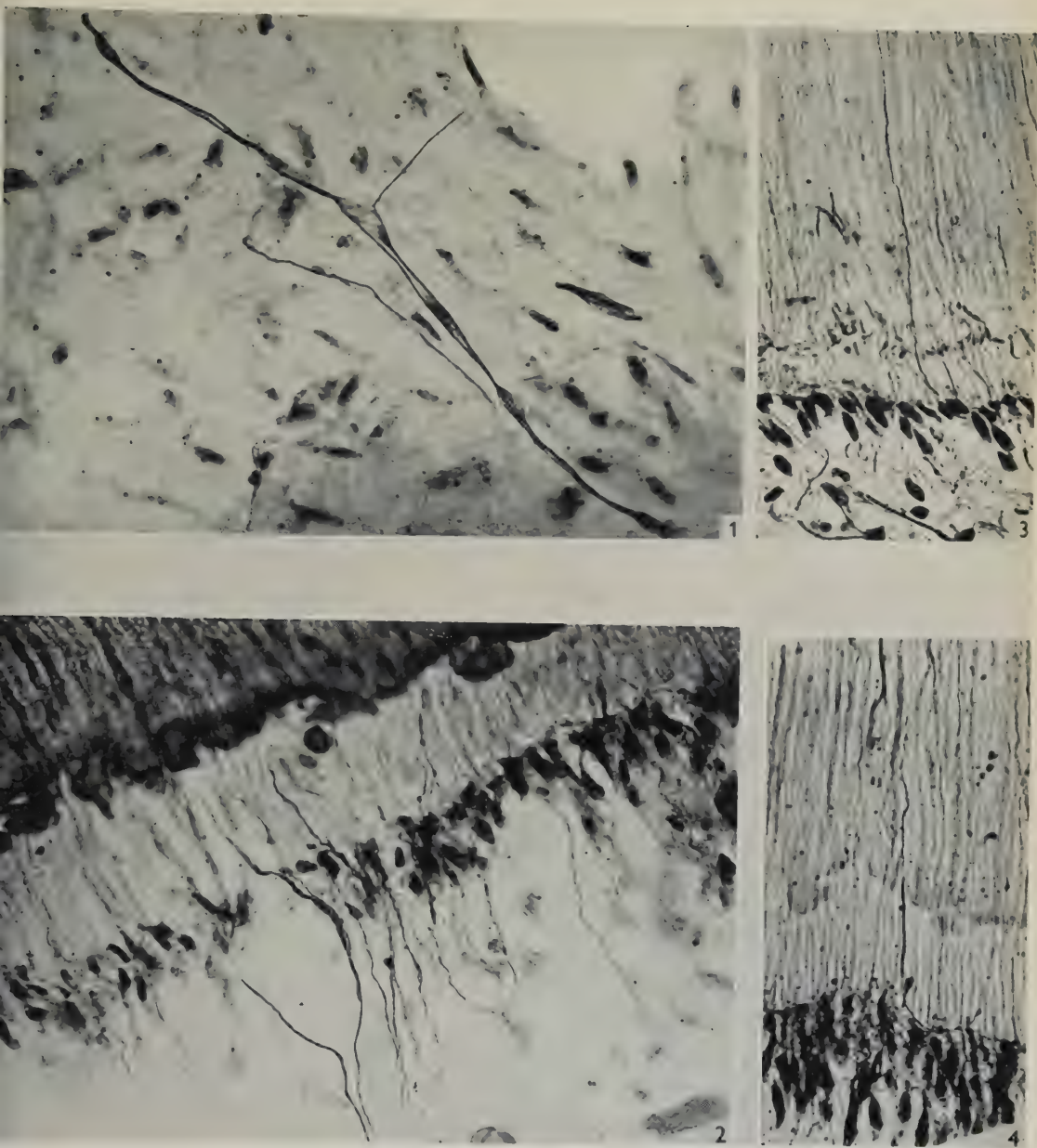


- TOJODA, M. (1934). Die Innervation des menschlichen Zahnbeins. *Dtsch. zahnärztl. Wschr.* **37**, 641-645, 670-673.
- UNGEWITTER, L. H. (1951). Urea silver nitrate method for nerve fibres and nerve endings. *Stain. Tech.* **26**, 73-76.
- WELLINGS, A. W. (1940). The entrance of nerves into the dentine. *Proc. R. Soc. Med.* **33**, 563-576.

## EXPLANATION OF PLATE

(All human material.)

- Fig. 1. Axons lying in the pulp showing apparent branching and varicosities.  $8\mu$  thick;  $\times 560$ .
- Fig. 2. Many fine axons passing from the pulp between the odontoblasts and into the predentine.  $25\mu$  thick;  $\times 560$ .
- Fig. 3. An axon passing from the pulp through the predentine and continuing deeply into the calcified dentine.  $8\mu$  thick;  $\times 469$ .
- Fig. 4. Another axon passing from the pulp through the predentine and continuing deeply into the calcified dentine.  $8\mu$  thick;  $\times 469$ .







# THE PERIVASCULAR SPACES OF THE MAMMALIAN CENTRAL NERVOUS SYSTEM AND THEIR RELATION TO THE PERINEURONAL AND SUBARACHNOID SPACES

BY D. H. M. WOOLLAM AND J. W. MILLEN

*Department of Anatomy, University of Cambridge*

Present concepts of the pathways followed by the cerebro-spinal fluid rest almost entirely on circumstantial evidence. The fluid, being, as Halliburton (1916) aptly put it, 'an ideal physiological saline', is not identifiable from the general tissue fluids by any known histochemical technique. Current text-book descriptions of its production, circulation and absorption therefore derive largely from experiments in which foreign substances were introduced into the cerebro-spinal fluid of animals and their distribution identified after the death of the animals. These descriptions are drawn chiefly from the conclusions of one particular group of experiments, those of Weed (1914*a, b*), using the Prussian blue technique. Indeed, if the principles underlying Weed's work were agreed to be erroneous, very little evidence would remain to support existing conceptions of the production, circulation and absorption of the cerebro-spinal fluid. Evidence for the production of the cerebro-spinal fluid by the choroid plexuses and its absorption by the arachnoid villi would be based almost entirely on the rather gross operative procedures which are associated with the name of Dandy and have been so severely criticized by Flexner (1933), and, in these circumstances, the details of its circulation would be almost completely unknown. It would appear therefore that, in the absence of better methods, the investigation of the circulation of the cerebro-spinal fluid must be based on the introduction of an indicator into the subarachnoid space and its subsequent identification by the histological study of the tissues.

The anatomy of the perivascular and perineuronal spaces forms one aspect of the circulation of the cerebro-spinal fluid in which current knowledge depends of necessity entirely on experiments of the type performed by Weed. There is an extensive literature bearing on these structures which has been reviewed elsewhere (Woollam & Millen, 1954). A study of this literature makes it clear how much of the confusion which surrounds the perivascular and perineuronal spaces to-day arose. It would appear that Weed himself was not primarily interested in the histology of these spaces but only in their possible physiological role. Consequently, he performed little detailed histological investigation, and based his conception of the anatomy of the perivascular and perineuronal spaces on the descriptions of earlier observers, notably on that of Mott (1910). It seems that later writers have drawn their descriptions of these spaces directly from Weed, and thus inadvertently perpetuated to the present day some of the early errors in description.

The present investigation was undertaken solely with the object of determining the relationship of the spaces in the outer coverings of cerebral and spinal blood vessels to the perineuronal spaces on the one hand, and the subarachnoid space on

the other. As an essential pre-requisite, it was necessary to decide which of the spaces concerned were artefact and which real spaces. No attempt, however, was made to investigate the details of the spatial distribution of the perivascular channels in the various parts of the central nervous system.

#### MATERIALS AND METHODS

In the first group of experiments, the technique used by Weed (1914*a*) and Patek (1944) was repeated in as much as the intravenous administration of hypertonic saline was employed in an attempt to facilitate the passage of indian ink injected into the subarachnoid space of an adult rat into the perivascular channels, thus rendering these channels easily identifiable in histological sections.

As a result of the experience gained in this first group of experiments, a new technique was evolved which differed from those previously employed in the following particulars:

- (1) The new-born rat was used as the experimental animal.
  - (2) The indicator was injected into the subarachnoid space in very small quantities (0.05 ml.) daily for periods of up to 3 weeks.
  - (3) The indicator chosen was 'dag' colloidal carbon (Acheson Colloids Ltd.) in which 99% of the particles were less than  $1\ \mu$  in diameter.
  - (4) Intravenous hypertonic saline was not administered.
- The experimental details in the two groups were as follows.

##### *Group 1. Experiments on adult rats*

Twenty-seven rats were used in the experiments in this group.

Ether was used as the anaesthetic and an injection of between 0.2 and 0.5 ml. of indian ink was made through a burr-hole in the parietal bone into the cranial subarachnoid space. The rat was allowed to recover and 2 hr. later, after again being anaesthetized with ether, received an injection of 4N. saline (prepared to the formula given by Weed and McKibben (1919)) intravenously. Two hours later the animal was killed by exsanguination under anaesthesia and the tissues fixed in 10% formalin. The brain and spinal cord were then removed intact from their bony coverings. Sections were cut and stained by a variety of methods including trichrome, haematoxylin and eosin, toluidine blue and a modification of the Bielschowsky stain. Most help was obtained from Long's (1948) reticular stain, modified by the addition of neutral red, which enabled the nerve cells to be seen in relation to the reticular perivascular sheath.

##### *Group 2. Experiments on new born rats*

All the operations were performed under conditions of strict asepsis. The members of a litter of rats were taken from the mother each morning from the day of their birth. Each young rat received an injection of 0.05 ml. of 'dag' colloidal carbon through the parieto-occipital suture into the cranial subarachnoid space. Before the plunger of the syringe was depressed, slight traction was exerted on it to remove a little cerebro-spinal fluid and mix it with the indicator so that the cerebro-spinal fluid pressure was not significantly elevated by the injection. Under these con-

ditions the rats thrived and the indicator appeared to circulate freely throughout all the spaces which the cerebro-spinal fluid was capable of entering. After a varying period of time, generally after 3 weeks of this treatment, the rats were sacrificed and preserved in formalin. Later the brains and spinal cords were removed, sectioned and stained as were those in group 1.

Of the forty-five rats used in this series, ten were eaten by the mother, ten died for unknown reasons, and twenty-five were killed deliberately at the end of the experiment. The brains and spinal cords of fifteen of these were examined histologically and all showed evidence of colloidal carbon in the perivascular spaces.

## RESULTS

Throughout the description of the findings the convention is adopted of employing the term 'true perivascular space' to denote that space which is in continuity with the general subarachnoid space.

The experiments in group 1 served the purpose of clarifying the relationships of the true perivascular spaces at their openings on the surface of the brain and spinal cord. The indian ink did not penetrate deeply into the perivascular spaces except in one situation and this was in relation to the basal ganglia.

When the brains and cords of the animals in group 2 were examined it was found that the colloidal carbon had passed along the cerebral and spinal arteries and veins to such an extent that it had, for example, reached the perivascular sheaths of the blood vessels in the substance of the ventral horn of the spinal cord. It was found that the indicator was generally to be seen only in relation to the larger branches of the arteries and tributaries of the veins. In some instances, however, quite small blood vessels were found to be surrounded by indicator in their perivascular spaces, while larger blood vessels were not similarly ensheathed (Pl. 1, fig. 1). It was impossible to determine whether this finding was merely due to the capricious behaviour of the indicator or did in fact represent a real difference between the coverings of the vessels concerned.

The examination of serial sections stained alternately with Long's stain for reticulum, Bielschowsky's stain and toluidine blue revealed that the carbon particles lay between the layers of the reticular perivascular sheaths of the blood vessels, and that they had penetrated to a point where the two layers of the sheaths appeared to unite to close off the perivascular channels. The illustration (Pl. 1, fig. 2) shows the colloidal carbon particles closely aggregated around a blood vessel in the ventral horn of the spinal cord. This would suggest that the large clear space surrounding the carbon particles was the true perivascular space, but examination of the next section in the series stained to show reticulum shows that the reticular perivascular sheath is closely applied to the vessel and contains the carbon particles. The large clear space was therefore regarded as an artefact. From the examination of the distribution of the carbon particles in relation to the reticular perivascular sheath, it was concluded that the larger vessels entering or leaving the brain and spinal cord had perivascular spaces which penetrated more deeply into the substance of the cord than did those accompanying smaller vessels.

The relationship of the true perivascular space to the subarachnoid space is



shown by the illustration (Pl. 1, fig. 3), of a blood vessel on the surface of the brain and a branch of that vessel entering the substance of the brain. The large blood vessel itself lies in a pia-arachnoid envelope completely filled with indian ink. The indian ink has penetrated for a short distance into the perivascular space surrounding the branch, and this perivascular space is separated from the substance of the brain by a space, traversed by trabeculae, which is clearly the result of shrinkage. This latter space is continuous with another space lying between the brain substance and the pia mater, and not with the subarachnoid space.

Trabeculae crossing the true perivascular space were never seen in the sections examined. They were, however, constantly seen in the artefact space external to the true perivascular space. In a section taken from the cerebrum of a rat which had received an injection of hypertonic saline 2 hr. before death, a space crossed by trabeculae was seen lying external to the space filled with indian ink (Pl. 1, fig. 4); clearly the former was a 'shrinkage' space produced by the effect of hypertonic saline. Careful comparison of the sections taken from animals which had received intravenous hypertonic saline before death with those which had not received such an injection showed that the principal effect of the injection was to dilate the artificial spaces lying between the blood vessels and the nervous tissue and to produce the trabeculated appearance of this system of spaces.

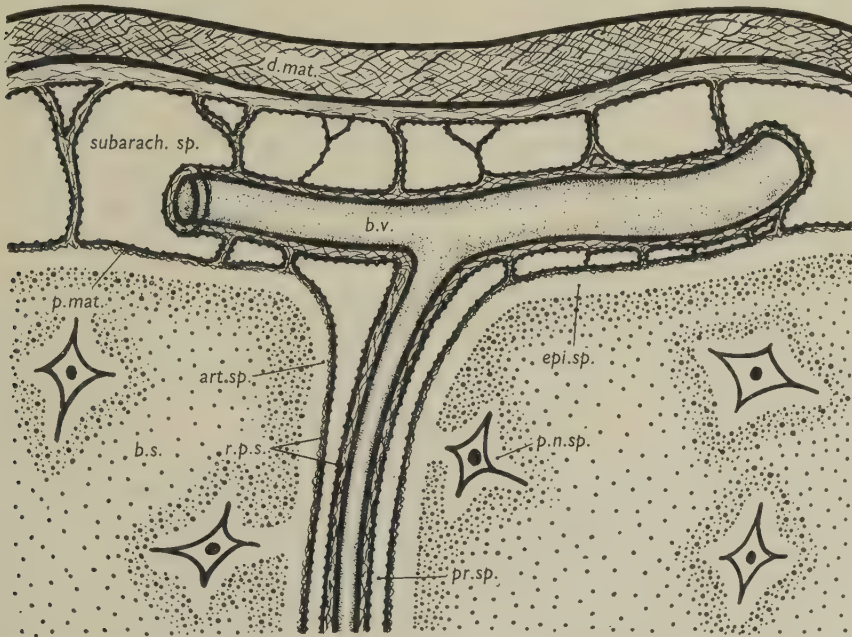
That this artefact space was not continuous with the true perivascular space was positively shown by the examination of the sections stained for reticulum with Long's stain. By this staining method the reticular perivascular tissue was sharply differentiated, and in these sections the clear artefact space around the blood vessel could easily be demonstrated to lie external to the sheath surrounding the true perivascular space, and the presence of the trabeculae crossing the artefact space could clearly be seen (Pl. 1, fig. 5).

By the use of Bielschowsky's stain it was demonstrated that the trabeculae were not processes of nerve cells, nor were they stained by the reticular stain. For these reasons it was assumed that earlier observers were correct in regarding them as glial fibres.

The relationship of the perineuronal space to the perivascular space was the object of considerable study. Occasionally an appearance was seen which suggested that the indicator had entered the perineuronal spaces. Careful examination, however, invariably revealed that where discrete carbon particles were seen in the perineuronal spaces they were present only in very small numbers and were scattered throughout the entire section in a manner suggesting that they had been dispersed over the surface of the section by the blade of the microtome knife. In many specimens this dispersion had not occurred and the perineuronal spaces contained either no carbon particles or only an occasional particle. Whenever a carbon particle was seen to lie in a perineuronal space in a section not covered by scattered carbon particles, it was always found that it lay within the substance of a macrophage. Further examination revealed that the perineuronal space was indeed continuous with a space surrounding a blood vessel, but not with the true perivascular space. It was continuous with the artefact space surrounding the reticular perivascular sheath, and lying between that sheath and the substance of the nervous tissue.

The foregoing observations may be summarized as follows (Text-fig. 1). The true

perivascular space lies between the two layers of a lepto-meningeal sheath continuous with the pia-arachnoid envelope of the central nervous system, and is continuous with the subarachnoid space in which the cerebro-spinal fluid circulates. It exists only around the larger blood vessels, although the arterioles, venules and larger capillaries all have a perivascular sheath. A second, artificial, space, produced by shrinkage of the brain and spinal cord after fixation and sectioning, can be seen between the perivascular sheath and the nervous tissue, and is continuous on the one hand with a space between the surface of the brain and the pia mater, 'the



Text-fig. 1. Diagram to illustrate the relationships of the perivascular space. *art.sp.*, artefact space; *b.s.*, brain substance; *b.v.*, blood vessel; *d.mat.*, dura mater; *epi.sp.*, epispinal space of His; *p.n.sp.*, perineuronal space; *pr.sp.*, perivascular space; *p.mat.*, pia mater; *r.p.s.*, reticular perivascular sheath; *subarach.sp.*, subarachnoid space.

epispinal space of His', and, on the other, with the perineuronal spaces. The results of the present experiments support the view that the perineuronal spaces and the epispinal space of His belong to the same system of artefact spaces as the space between the perivascular sheath and the nervous tissue. All these artefact spaces are produced by shrinkage, and there appears to be no reason to suppose that they have any significance in the normal structure and function of the central nervous system.

#### DISCUSSION

The sense in which the term 'perivascular space' is understood by modern writers appears to be that of a space surrounding the blood vessel, which communicates freely with the general subarachnoid space in which the cerebro-spinal fluid circulates. The findings of the present investigation confirm that such a space exists and

that it is possible for particulate matter introduced into the subarachnoid space to enter this perivascular space without the tonicity of the blood being altered to bring this about. It is therefore reasonable to suppose that all those authors who have used the introduction of an indicator into the subarachnoid space to outline the perivascular space have described a space which corresponds to the one identified as the 'true' perivascular space in the description above, with the proviso that those who have used unphysiological pressures of injection or unsuitable indicators may have obtained a false picture of its extent and connexions.

For these reasons the contributions of the earlier observers, based as they were upon the histological examination of normal and pathological material without the aid of experimental methods, are now of historical interest only. Although the spaces were first described by Pestalozzi (1849), they are often given the name of 'Virchow-Robin' spaces from their description by Virchow (1851) and Robin (1859). It is interesting to note that these two authorities were not in agreement as to the situation and relations of the spaces, for Virchow considered that the spaces lay between the tunica media and tunica adventitia of the blood vessels, and were freely open to the subarachnoid space, while Robin regarded the spaces as closed at both ends and intra-adventitial in situation.

His (1865) was the first to apply experimental methods to the solution of this problem. He introduced ink by means of a multiple puncture technique directly into the substance of the brain. He was unaware of the existence of the reticular perivascular sheath which was described for the first time by Key & Retzius (1876), and regarded the spaces which he described as 'lymphatics' connecting with the perineuronal spaces. It may be argued that, if an injection be made directly into the substance of the brain, the chances of the indicator entering the true perivascular spaces would be extremely small. However, there is every possibility that it will be found in the artificial shrinkage spaces mentioned above. Even an injection intended for the subarachnoid space may accidentally pierce the pia mater and progress by opening up the whole system of artefact spaces. It is highly probable that it was some such combination of circumstances which led later writers (e.g. Bevan Lewis, 1889) to believe that the perivascular space was continuous with the perineuronal space.

In Weed's (1923) description, the perivascular spaces were stated to extend along the artery, arteriole, capillary, venule and vein, and to communicate freely not only with the perineuronal spaces but also with potential spaces between the glial elements and fibre tracts of the brain and spinal cord. The latter is an aspect of Weed's findings which has not been stressed by those text-books which otherwise adopt his descriptions of the perivascular spaces. To accept Weed's account of the connexions of the perivascular spaces is tantamount to an admission that no obstruction is offered by the perivascular sheaths to the passage of the cerebro-spinal fluid. Since the morphology of these sheaths is identical with that of the pia-arachnoid layers which contain the cerebro-spinal fluid (Key & Retzius, 1876), such a conception would appear to contradict the view that the membranes which surround the subarachnoid space in any way exert a retentive effect on the cerebro-spinal fluid. The method which Weed devised (Weed, 1914*a, b*, 1923), was a combination of the subarachnoid injection of a double salt of potassium ferrocyanide and iron ammo-



nium citrate with the intravenous administration of hypertonic saline in the living animal. Weed considered that the use of hypertonic saline, by altering osmotic pressures, facilitated the penetration of the double salt along the perivascular channels. When the brain and spinal cord were later removed and treated with acid formalin, Prussian blue particles were deposited wherever the double salt had penetrated. Weed made the assumption that the normal passage of the cerebro-spinal fluid corresponded to that of the double salt in his experiments. It is most significant to note that when Weed himself used indian ink as an indicator, he failed to obtain anything other than what he regarded as 'partial' filling of the perivascular spaces. It is clear that Weed drew his conceptions of the histological relationships of the perivascular spaces largely from the papers of Tuke (1894) and Mott (1910), both of which accounts seem to derive to a considerable extent from Bevan Lewis (1889) who erroneously identified the space injected by His with the true perivascular space of Virchow-Robin. The results of the present investigation, together with those described by Patek (1944), indicate that the perineuronal space is an artefact and the idea that it communicates with the perivascular space should be discarded.

#### SUMMARY

Because of the complexity of the tissue layers involved and the difficulty in obtaining a suitable indicator which, injected into the subarachnoid space, serves to outline the perivascular spaces, the accounts in the literature of these spaces are confused and contradictory.

In the present investigation, two groups of experiments were performed. In the first group, the single injection of indian ink into the subarachnoid space of the adult rat was followed 2 hr. later by the intravenous administration of hypertonic saline. In the second group, the subarachnoid injection of colloidal carbon daily in the new-born rat from birth to about 3 weeks of age served to outline the perivascular spaces. In both groups the brain and spinal cords were removed, fixed, sectioned, stained and examined histologically. It was found that there are two spaces surrounding the blood vessel in the central nervous system. The first is the true perivascular space which communicates with the subarachnoid space but not with the perineuronal spaces. External to this true perivascular space lies an artefact space which communicates with the perineuronal spaces and with the epispinal space of His between the pia mater and the surface of the brain and spinal cord, but not with the subarachnoid space. All these spaces are artefacts, the results of shrinkage consequent to fixation and sectioning.

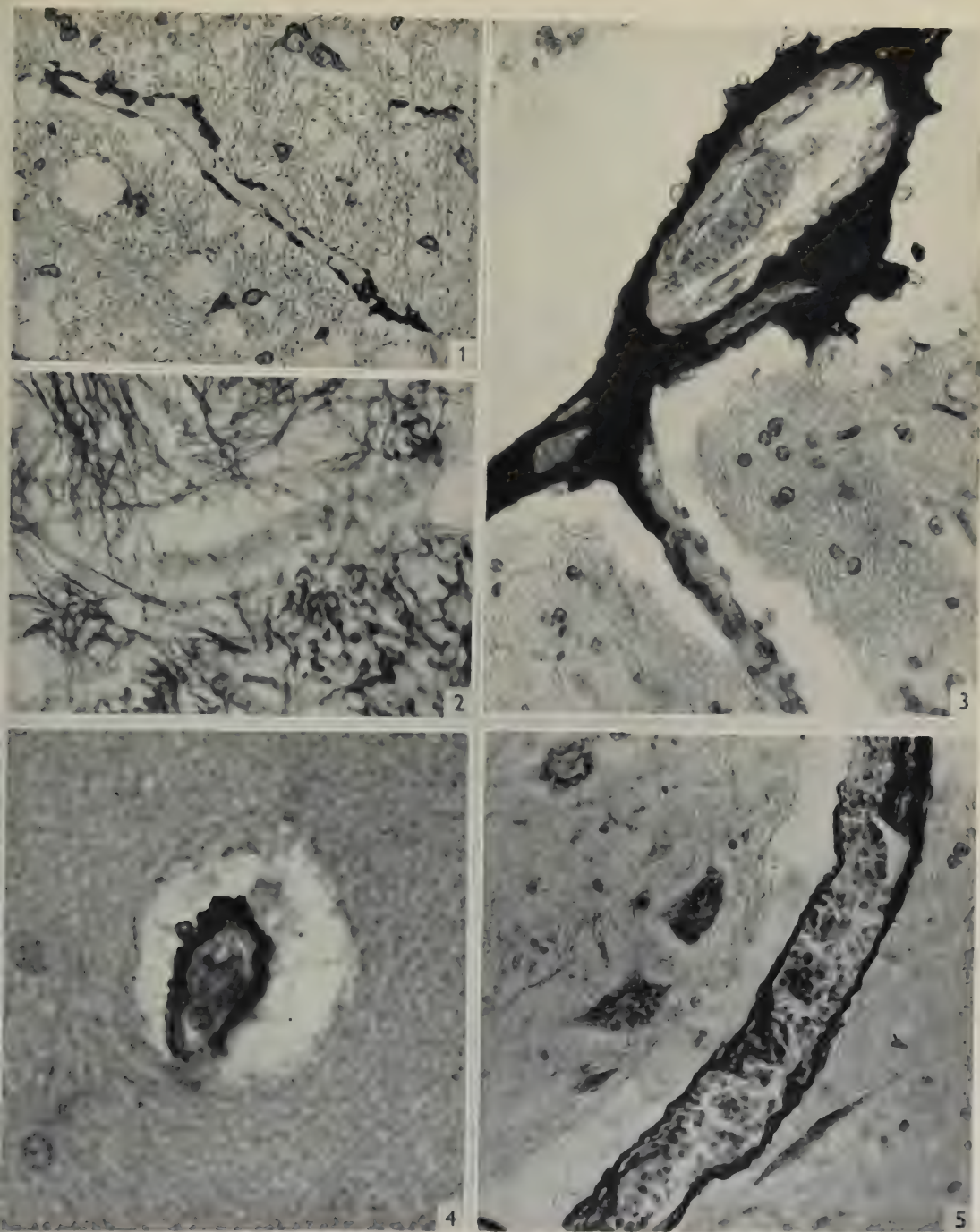
We are greatly indebted to Prof. J. D. Boyd for his advice in the preparation of this paper. We also wish to express our gratitude to Mr T. R. L. Brooks for the photographic work and to Mr R. Smith for technical assistance.

## REFERENCES

- BEVAN LEWIS, W. (1889). *A Text-book of Mental Diseases with Special Reference to the Pathological Aspects of Insanity*, p. 81. London: Charles Griffin.
- FLEXNER, L. B. (1933). Some problems of the origin, circulation and absorption of cerebrospinal fluid. *Quart. Rev. Biol.* **8**, 397-422.
- HALLIBURTON, W. D. (1916). The possible functions of the cerebro-spinal fluid. Presidential Address. Section of Neurology. *Proc. R. Soc. Med.* pp. 1-12.
- HIS, W. (1865). Über ein perivasculäres Canalsystem in den nervösen Centralorganen und über dessen Beziehungen zum Lymphsystem. *Z. wiss. Zool.* **15**, 127-141.
- KEY, A. & RETZIUS, G. (1876). *Studien in der Anatomie des Nervensystems und des Bindegewebes*. Stockholm.
- LONG, MARGARET E. (1948). Differentiation of myofibrillae, reticular and collagenous fibres in vertebrates. *Stain Tech.* **23**, 69-75.
- MOTT, F. W. (1910). The Oliver Sharpey Lectures on cerebrospinal fluid. *Lancet*, **2**, 1-8.
- PATEK, P. R. (1944). The perivascular spaces of the mammalian brain. *Anat. Rec.* **88**, 1-13.
- PESTALOZZI, R. (1849). Über Aneurysmata spuria der kleinen Gehirnarterien und ihren Zusammenhang mit Apoplexie. Würzburg: F. E. Thein.
- ROBIN, C. (1859). Recherches sur quelques particularités de la structure des capillaires de l'encéphale. *J. Physiol. l'homme*, **2**, 536-548.
- TUKE, J. B. (1894). Morison Lectures on insanity. *Edinb. med. J.* **39**, 673-683.
- VIRCHOW, R. (1851). Über die Erweiterung kleinerer Gefäße. *Virchow's Arch.* **3**, 427-462.
- WEED, L. R. (1914a). Studies on cerebro-spinal fluid. No. II. The theories of drainage of cerebro-spinal fluid with an analysis of the methods of investigation. *J. med. Res.* **31**, 21-49.
- WEED, L. H. (1914b). Studies on cerebro-spinal fluid. No. III. The pathways of escape from the subarachnoid spaces with particular reference to the arachnoid villi. *J. med. Res.* **31**, 51-91.
- WEED, L. H. (1923). The absorption of cerebro-spinal fluid into the venous system. *Amer. J. Anat.* **31**, 191-221.
- WEED, L. H. & MCKIBBEN, P. S. (1919). Pressure changes in the cerebro-spinal fluid following intravenous injection of solutions of various concentrations. *Amer. J. Physiol.* **48**, 512-530.
- WOOLLAM, D. H. M. & MILLEN, J. W. (1954). Perivascular spaces of the mammalian central nervous system. *Biol. Rev.* **29**, 251-283.

## EXPLANATION OF PLATE 1

- Fig. 1. Section through spinal cord of young rat which received a daily subarachnoid injection of colloidal carbon from birth to the age of 20 days when it was killed. Toluidine blue stain,  $\times 435$ . The section shows carbon particles in the perivascular space of a small blood vessel in the region of the posterior horn.
- Fig. 2. Section through spinal cord of young rat which received a daily subarachnoid injection of colloidal carbon from birth to the age of 21 days when it was killed. Bielschowsky's stain,  $\times 500$ . The section shows carbon particles aggregated on and around a blood vessel in the anterior horn region of the cord. The clear space surrounding the blood vessel is the artefact space.
- Fig. 3. Section through cerebrum of rat which received a subarachnoid injection of indian ink 4 hr. and an intravenous injection of hypertonic saline two hours before being killed by exsanguination. Haematoxylin and eosin stain,  $\times 400$ . The indian ink surrounding the large blood vessel on the surface of the cerebrum lies in the subarachnoid space. The clear space between the ink and the brain substance is the epispinal space of His. The ink has penetrated into the perivascular space of the vessel entering the substance of the cerebrum. The space crossed by trabeculae external to the perivascular space is the artefact space.
- Fig. 4. Section through cerebrum of rat which received a subarachnoid injection of indian ink 4 hr. and an intravenous injection of hypertonic saline 2 hr. before being killed by exsanguination. Haematoxylin and eosin stain,  $\times 875$ . The indian ink surrounding the blood vessel lies in the perivascular space. The trabeculated space external to this is the artefact space.
- Fig. 5. Section through spinal cord of young rat. Modification of Long's reticular stain,  $\times 435$ . The perivascular sheath of the blood vessel is surrounded by a clear space crossed by trabeculae, the artefact space.



WOOLLAM AND MILLEN - PERIVASCULAR SPACES OF THE MAMMALIAN CENTRAL NERVOUS SYSTEM

(Facing p. 200)





# OBSERVATIONS ON THE DEVELOPMENT OF MICROGLIA TOGETHER WITH A NOTE ON THE INFLUENCE OF CORTISONE

BY E. J. FIELD

*Anatomy Department, University of Bristol*

Since Hortega's studies some thirty years ago it has been widely accepted that microglia is of mesodermal origin and makes its appearance in the central nervous system during the last stages of intrauterine development ('en los ultimos periodos del desarrollo embrionario'), being especially conspicuous round about the time of birth. At this stage 'fountains' of immature microglial cells are to be seen invading the brain in certain well-defined localities such as the root of the tela choroidea. Hortega's definitive views, together with an extensive survey of the literature, have been set out in his contribution to Penfield's *Cytology and Cellular Pathology of the Nervous System* (1932). However, in the same year it was claimed that mature microglia could be demonstrated in the early embryonic brain and that the compound granular corpuscle type of cell characteristic of Hortega's fountains only appeared late in development (Santha, 1932). Other workers, whilst confirming Hortega's observations in the late embryonic and neonatal stages, have offered different accounts of the earliest appearance of microglia (Santha & Juba, 1933; Juba, 1934; Metz & Spatz, 1924). Pruijus (1927) stands apart in being unable to confirm Hortega's observations and in his suggestion of an ependymal (i.e. neuroectodermal) origin for microglia.

The present work is concerned mainly with the more controversial question of the early appearance of microglia. In general, all Hortega's actual observations (so far as they went) have been confirmed in the present material of comparable age and will not be described here.

## MATERIAL AND METHODS

Mouse, rat, human, sheep and goat material has been examined. In all except the human, the examination has been carried into the postnatal stage. Thirty mouse, eighty-one rat, twenty-three human, ten sheep and four goat specimens have been used. In addition, forty-eight rats have been injected with 2.5 or 5 mg. doses of cortisone per day subcutaneously over a 1- to 3-day period beginning shortly after birth. Only the central nervous system changes in these animals will be described here. The method of Hortega has been employed after fixation in Cajal's formol ammonium bromide mixture. Fat staining has been carried out by the Herxheimer, Globus and Gross methods, as well as by Sudan black. Paraffin sections stained by Giemsa have also been examined.

## OBSERVATIONS

*Mouse*

Well developed and branched microglial cells can be seen at the 1.3 cm. stage (Pl. 1, fig. 1), but they are few in number, often only one or two appearing in a single section. In general, the branching is relatively coarse with short, thick, secondary processes. No special relation to blood vessels was noted. At 16 mm. such cells were a little more frequent but often quite isolated (Pl. 1, fig. 2) and similar strongly argyrophilic cells were to be seen elsewhere in the head, e.g. in the tongue and subcutaneous tissues. Occasional microglial cells containing ingested granular material were to be found from about the 23 mm. stage onward (Pl. 1, fig. 3) so that many cells appeared to have coarsely granular processes. At birth, and in the neonatal period, there were numbers of intricately branched cells (Pl. 1, fig. 4), but after about the fifth day they became progressively less easy to impregnate. At 18 days, microglia of the type seen in Pl. 1, fig. 4 was no longer found but considerable numbers of large cells of oligodendroglial type appeared (Pl. 1, fig. 5). Their origin was uncertain but thenceforth they dominated the picture, becoming gradually smaller and more fully stainable (Pl. 1, fig. 6). 'Fountains' of microglia were very poorly developed, only a few rather foamy cells being seen in the corpus callosum and its radiation into the hemisphere. Soon after birth large numbers of very small cells with short thick processes began to take on the silver stain. They were probably astroglia, though they could not be impregnated by the gold chloride method. Adult microglia in the mouse is a relatively simple cell type (Pl. 1, fig. 7) possessing few processes and devoid of complicated branchings.

*Rat*

The earliest specimen examined (9 mm.) showed already commencing vascularization of the cerebral hemispheres as well as other parts of the nervous system. On the walls of cerebral capillaries deeply staining cells could be seen here and there, sometimes possessing delicate processes. In addition, similar branched cells were located free from vessels (Pl. 1, fig. 8). Similar deeply staining cells were present here and there within blood vessels. Such appearances suggest an emigration of early microglioblasts from incoming blood vessels. The cells do not go through a compound granular corpuscle stage but become directly more complicatedly and finely branched. At this stage there are no foamy cells beneath the covering membranes of the brain (Pl. 1, fig. 9).

Occasionally islands of cells are encountered here and there in the nervous system (Pl. 1, fig. 10). In such islands there are many cells containing granular inclusions. They seem to be nests of microglioblasts. Careful examination suggests that they are not artefact extravasations and they resemble the accumulations described by Juba (1934).

Within the spinal cord well-branched microglial cells and many lamellar cells are present by the 19 mm. stage (Pl. 1, fig. 11). Large numbers of amoeboid cells, many containing ingested fragments, are present at this stage elsewhere in the body, especially in the connective tissue planes between muscles (Pl. 2, fig. 12).

From about this stage, too, rounded amoeboid cells with granular cytoplasm (but



not containing any sudanophilic material) begin to appear in the brain. Sometimes they occur in localized collections (Pl. 2, figs. 13, 14), but more consistently they are to be found in the thickness of the cerebral hemisphere, about the lateral angles of the fourth ventricle and around the cavity of the midbrain. Their localization in general is to regions where rapid form alterations are taking place or where a 'sliding plane' of adjustment between external and internal configurational changes might be expected. This suggests their form may be associated with some alteration in the physico-chemical constitution of the ground substance of the brain in these situations, since the form of a cell is influenced by the character of its milieu. These rounded cells replace fully branched microglial cells in the special locations mentioned, and the bands of granular rounded cells stretching as a layer-like formation in the cerebral hemisphere (Pl. 2, fig. 15) are the forerunners of the main 'fountain' described by Hortega, being recognizable in the rat by the 19 mm. stage.

At birth (3.0–3.5 cm.) 'fountains' are well developed and are of the appearance fully and accurately described by Hortega. They remain prominent until about the 5th or 8th day, though small numbers of amoeboid cells can be found at the ends of the callosal radiation up to about the end of the first month, whilst matured microglia is present along the course of the radiations. In the first few days after birth, Hortega's method as practised by the author brings out fine beaded fibres which turn up into the cortex from the white matter (Pl. 2, fig. 16). The nature of these fibres is not clear. They pass right up into the cortex, becoming progressively finer and may well be some form of sustentacular fibre. In adult brains such fibres do not stain by Hortega's method, but microglia itself is readily impregnated and resembles that seen in the rabbit.

Exhibition of cortisone to neonatal animals in the high doses mentioned above leads to a suppression of Hortega's fountains (Pl. 2, figs. 17, 18) and also considerable delay in myelination. A reduced number of microglial cells are present in the radiation of treated animals but are not of amoeboid form, being branched and more mature in appearance. These findings suggest that cortisone has impeded the migration and phagocytic activity of microglia which has reverted to the branched inactive form (Pl. 2, fig. 19; Pl. 3, fig. 20). These changes are part of a striking general picture of developmental inhibition apparently due to pituitary hypofunction (Field, 1954*a*).

#### *Human*

In the earliest specimen examined by Hortega's method (5.5 cm.) isolated well-matured microglial cells were found in the internal capsule region, in the spinal cord and in the medulla. These cells were frequently widely isolated from one another and there was no evidence of origin from blood vessels (Pl. 3, fig. 21). Sometimes isolated giant cells occurred, especially in the midline raphe of the medulla (Pl. 3, figs. 22, 23). In general, the processes of early mature microglial cells extended for much greater distances than those of adult cells. Amoeboid cells were present beneath the ependyma of the midbrain (Pl. 3, fig. 24) and also in the internal capsule (Pl. 3, figs. 25, 26). Despite their appearance they contained no sudanophilic material.

From the 15 cm. stage islands of intensely argyrophilic cells appeared in the cerebral cortex (Pl. 3, fig. 27). These islands are quite different in character from the

islands described above in the rat brain. Cells of microglial morphology were seen entangled in masses of deeply staining fibres which passed for considerable distances through the thickness of the cortex. At later stages these islands become less numerous, and individual cells detach themselves from the main mass to become independent (Pl. 3, fig. 28). Cajal's gold chloride method for astrocytes does not stain these cells.

#### Sheep

In the early sheep embryo (2.6 mm.) rounded and tuberoso cells were often seen in the vicinity of blood vessels in the cerebral hemisphere and elsewhere in the nervous system (Pl. 3, fig. 29). Some cells had already become complicatedly branched at this stage (Pl. 3, fig. 30). Amoeboid cells were found in the root of the choroid plexus at 23 mm.

Branched microglia became more numerous up to about the 18 cm. stage, but after this no satisfactory demonstration of them could be made. Islands of cells similar to those described in the human cortex were occasionally met with. On the other hand, oligodendroglial cells became very readily stainable in the mature foetus.

#### Goat

No demonstration of microglia was possible in four neonatal animals examined, though astro- and oligo-dendroglia were readily impregnated.

### DISCUSSION

Hortega's views on the development of microglia, though still widely held, have been amended by some authors and denied altogether by others. The main features of his account are: (1) mesodermal origin of microglia; (2) its late appearance in ontogeny; and (3) its morphogenesis through an obligatory stage of foamy cell. This latter cell type Hortega subdivided into four classes: *formas redondeadas*, *formas amiboides*, *formas pseudopodicas* and *formas ramificadas*, constituting a cycle of obligatory evolution of microglia in late embryonic and early neonatal life. From 'fountains' of such cells of all types, invasion of the grey matter of the cortex and basal ganglia takes place. In general, these fountains originate in regions where cerebral tissue is in contact with covering mesodermal membranes, e.g. the root of the tela choroidea, the base of the cerebral peduncles. Gozzano (1929, 1931) confirmed Hortega's findings in neonatal dogs, cats and rabbits. He found many immature microglial cells in the white matter and more mature types in the deeper parts of the cortex. In the more superficial part of the cortex he found numerous foamy cells. He carefully refrained from drawing a firm conclusion as to the origin of microglioblasts: 'Non ho visto finora l'emigrazione di tale elementi delle parete dei vasi, come non l'ho visto dalle meningi' (1929). The same caution is exercised by Metz & Spatz (1924), who draw attention to Hortega's plates I-V, figs. 1-6 (1921) where '*Gitterzellen*' are illustrated immediately under the pia but not a single one is found actually in the membrane itself. Hortega, in his text, admitted that he did not actually see cells coming in from the pia and offered only general observations on a hypothetical origin from that membrane. Metz & Spatz, whilst confirming in general Hortega's actual descriptions, could not subscribe to his conclusion as to the mesodermal origin of microglia, but thought it a special form of neuroglia.

Pruijus (1927), working with neonatal rabbit material, concluded that microglia was derived from ependyma and was therefore of neuroectodermal origin passing through a '*Stäbchenzell*' phase before reaching maturity. He could not find the amoeboid globular forms described by Hortega. Rydberg (1932) supported Pruijus's views of the neuroectodermal origin of microglia in kittens and the human newborn.

In the same year as Hortega's definitive views were published (1932), reports appeared which, while confirming his observations as far as they went, extended them materially to the early stages of development. Thus, Belezky showed that amoeboid cells appear in the central nervous system of the chick at the fourth day of incubation. Santha (1932) demonstrated well-branched microglia in the cortex and white matter of the 31 and 38 cm. human foetus. Globose and tuberosa forms were not encountered, but in the callosal radiation plump, branched cells were seen. From his examination of this material and also rabbit and rat embryos he concluded that the '*Gitterzell*' type in Hortega's neonatal fountains did not occur during the first half of gestation, though well-matured forms were already present at this stage. Moreover, he found that the occurrence of microglia ran parallel with vascularization and general development of the region concerned, and concluded that a vascular origin of microglia was probable. Santha & Juba (1933), concentrating upon the rat, went on to show that cells of compound granular corpuscle type were found in the second half of gestation and that microglia during the early part of foetal life did not evolve through a '*Körnchenzell*' stage. They agreed with Berluchi (1930) that mechanical factors alone were not responsible for the occurrence of amoeboid cell types round about the time of birth, but some metabolic activity of the white matter might be concerned (see below).

Juba (1933) was able to identify amoeboid microglial cells in the 23 mm. human embryo derived apparently from dispersed rounded or polygonal electively stained cells similar in character to certain of the blood elements. He traced intermediary forms between these and mature microglia, which he therefore regarded as originating from a haematogenous mesodermal source. Again he emphasized the relationship between the first occurrence of vascularization and the appearance of microglioblasts. In 1934 Juba went on to extend Belezky's observations on the chick embryo, showing that no Hortega cells were to be found before vascularization had occurred, that the earliest rounded elements appeared in the vicinity of the incoming capillaries, and that such early forerunners of microglia occurred occasionally in clumps giving the appearance of a local lesion.

The early presence of microglia in the human brain has been confirmed by Kershman (1939) in the 8-week embryo. He recorded the presence of fat-containing amoeboid cells (Herxheimer method) at 12 weeks. Sudanophilic material, though carefully looked for, has been notably absent in the author's material even at late stages when myelination has already begun. Kershman found no evidence that microglia played any part in the process of myelination, though like earlier authors he found a constant association of microglial fountains with areas of tract formation suggesting the likelihood of an important functional correlation. Apart from this signal failure to demonstrate sudanophil material during foetal life the author's results confirm those of Santha, Juba and Kershman.



Additional evidence for the mesodermal origin of microglia and its invasion *en masse* around the time of birth is afforded by the cortisone-treated rats. Of the very numerous effects which cortisone may produce, three may be of particular significance in the present connexion: (1) a diminution in cellular migration from capillaries; (2) diminished amoeboid activity (Paff & Stewart, 1953); and (3) some alteration in the physico-chemical character of the ground substance of the brain in which presumably all cells, nervous and non-nervous, are embedded. (It is of course always possible that the glia is really a syncytium—as maintained by Held and his supporters—and that no ground substance exists as a distinct element.) These factors might well account for the very small numbers of microglia and the absence of active amoeboid forms in the cortisone-treated neonatal rat in the same way as they may be invoked to explain the diminished reaction in the vicinity of a stab wound of the brain in a cortisone-treated animal (Field, 1954).

An association between the presence of rounded, amoeboid, fat-laden cells of compound granular corpuscle type and the process of myelination has long been postulated (Boll, 1874; Eichorst, 1875) following Virchow's description of encephalitis interstitialis neonatorum in 1867. Hortega (1932), after an extensive review of Virchow's encephalitis, concluded that '...the fat granule cells cannot be considered as abnormal forms, since the microglia from the moment it enters the central nervous system and begins its phagocytic action may show cytoplasmic inclusions and may also produce lipoids' (p. 494). The foamy type of cell met with in the present work did not contain sudanophilic material, and in this respect resembled the pseudo-compound granular corpuscles or mulberry cells found in the early stages of reaction around stab wounds of the brain (Field, 1954). In both cases these cells may be handling some myelin intermediary product. If this be so, the appearance of Hortega's 'fountains' may result from phagocytic processes associated with myelination.

#### SUMMARY

1. The presence of mature microglia in early stages of embryonic development has been confirmed for mouse, rat, sheep and human. None has been demonstrated at late stages in the sheep.
2. Foamy microglial cells become prominent late in development. They do not contain sudanophilic material. Their possible relation to the process of myelination is briefly discussed.
3. Cortical islands or nests of (?) microglial cells have been described in the sheep and human brain.
4. Cortisone suppresses Hortega's neonatal microglial 'fountains'. The significance of this is discussed.
5. Microglia probably arises from an early blood element and is of mesodermal origin.

The author would like to thank Prof. J. M. Yoffey for laboratory facilities provided, and Miss C. M. Le Bechée for help with the histological preparations. He is indebted to the Medical Research Council for cortisone and the work has been done with financial help from the Council.

## REFERENCES

- BELEZKY, W. K. (1932). Über die Histogenese der Mesoglia. *Virchows Arch.* **284**, 295–311.
- BERLUCHI, C. (1930). Sulle cellule granulo grasse dei neonati. *Riv. Pat. nerv. ment.* **35**, 147–150.
- BOLL (1874). Cited by Hortega (1932).
- EICHORST, H. (1875). Ueber die Entwicklung des menschlichen Rückenmarkes und seiner Formelemente. *Virchows Arch.* **64**, 425–475.
- FIELD, E. J. (1954). Observations on the reaction of the mouse brain to trauma and a note on the influence of cortisone. *Proc. Anat. Soc., J. Anat., Lond.*, **88**, 556.
- FIELD, E. J. (1954a). Effect of cortisone on the neonatal rat. *Nature, Lond.*, **174**, 182.
- GOZZANO, M. (1929). Ricerche sull' isotogenesi della microglia. *Boll. Soc. ital. Biol. sper.* **4**, 1028–1031.
- GOZZANO, M. (1931). Sopra speciali corpi globosi osservati nel cervello di mammiferi neonati, et sui loro rapporti con i microglioblasti. *Boll. Soc. ital. Biol. sper.* **6**, 12–14.
- HORTEGA, P. DEL RIO (1921). Histogénesis y evolución normal; éxodo y distribución regional de la microglia. *Arch. Neurobiol.* **2**, 212–255.
- HORTEGA, P. DEL RIO (1932). Microglia: in Penfield's *Cytology and Cellular Pathology of the Nervous System*, vol. II. New York: Hoeber.
- JUBA, A. (1933). Untersuchungen über die Entwicklung der Hortegaschen Mikroglia des Menschen. *Arch. Psychol., N.Y.*, **101**, 577–592.
- JUBA, A. (1934). Das erste Ercheinen und die Urformen der Hortegaschen Mikroglia im Zentralnervensystem. *Arch. Psychol., N.Y.*, **102**, 225–232.
- KERSHMAN, J. (1939). Genesis of microglia in the human brain. *Arch. Neurol. Psychiat., Lond.*, **41**, 24–50.
- METZ, A & SPATZ, H. (1924). Die Hortegaschen Zellen (das sogenannte 'dritte Elemente') und über funktionelle Bedeutung. *Z. ges. Neurol. Psychiat.* **89**, 138–170.
- PAFF, G. H. & STEWART, R. (1953). Free wandering cells and cortisone. *Proc. Soc. exp. Biol., N.Y.*, **83**, 591–592.
- PRUIJUS, W. M. (1927). Über Mikroglia, ihre Herkunft, Funktion und ihr Verhältniss zu anderen Gliaelementen. *Z. ges. Neurol. Psychiat.* **108**, 298–331.
- RYDBERG, E. (1932). Cerebral injury in newborn children consequent on birth trauma; with an enquiry into normal and pathological anatomy of neuroglia. *Acta path. microbiol. scand.* (Suppl.), **10**, 1–247.
- SANTHA, K. VON (1932). Untersuchungen über die entwicklung der Hortegaschen Mikroglia. *Z. ges. Neurol. Psychiat.* **96**, 36–67.
- SANTHA, K. VON & JUBA, A. (1933). Weitere Untersuchungen über die Entwicklung der Hortegaschen Mikroglia. *Z. ges. Neurol. Psychiat.* **98**, 598–613.
- VIRCHOW, R. (1867). Zur pathologischen Anatomie des Gehirns. I. Congenitale Encephalitis und Myelitis. *Virchows. Arch.* **38**, 129–138.

## EXPLANATION OF PLATES

(Frozen sections 30–35 shown throughout.)

## PLATE 1

- Fig. 1. Section through basal ganglia of 13 mm. mouse embryo. Well-branched microglia present not showing special relation to capillaries. Hortega,  $\times 325$ .
- Fig. 2. Isolated bipolar microglial cell in ependyma of lateral ventricle of 16 mm. mouse embryo. Hortega,  $\times 325$ .
- Fig. 3. Microglia with coarse branched processes. A dark mass is included within the cytoplasm of the central cell. 23 mm. embryo mouse. Hortega,  $\times 325$ .
- Fig. 4. Five-day-old mouse; basal ganglia. Well-matured microglia present. Hortega,  $\times 400$ .
- Fig. 5. Eighteen-day-old mouse; basal ganglia. Cells of oligo-dendroglial type are brought out by Hortega staining. No microglia seen under ordinary conditions.  $\times 325$ .
- Fig. 6. Oligodendroglia of normal adult mouse. Hortega,  $\times 325$ .
- Fig. 7. Normal microglia of adult mouse (Ammon's horn).  $\times 510$ .
- Fig. 8. 10 mm. rat embryo, cerebral vesicle. Three deeply staining cells are seen applied to the wall of capillaries. Those to the right and left are provided with delicate processes. Below are two free cells. Hortega,  $\times 325$ .

- Fig. 9. 10 mm. rat embryo, cerebral vesicle. Note branched cells in superficial part of the cortex. Hortega,  $\times 325$ .
- Fig. 10. 13 mm. rat embryo, cervical cord. Focus of rounded amoeboid cells near surface. Hortega,  $\times 325$ .
- Fig. 11. 19 mm. rat embryo, cervical cord. Lamellar microglia present. Hortega,  $\times 600$ .

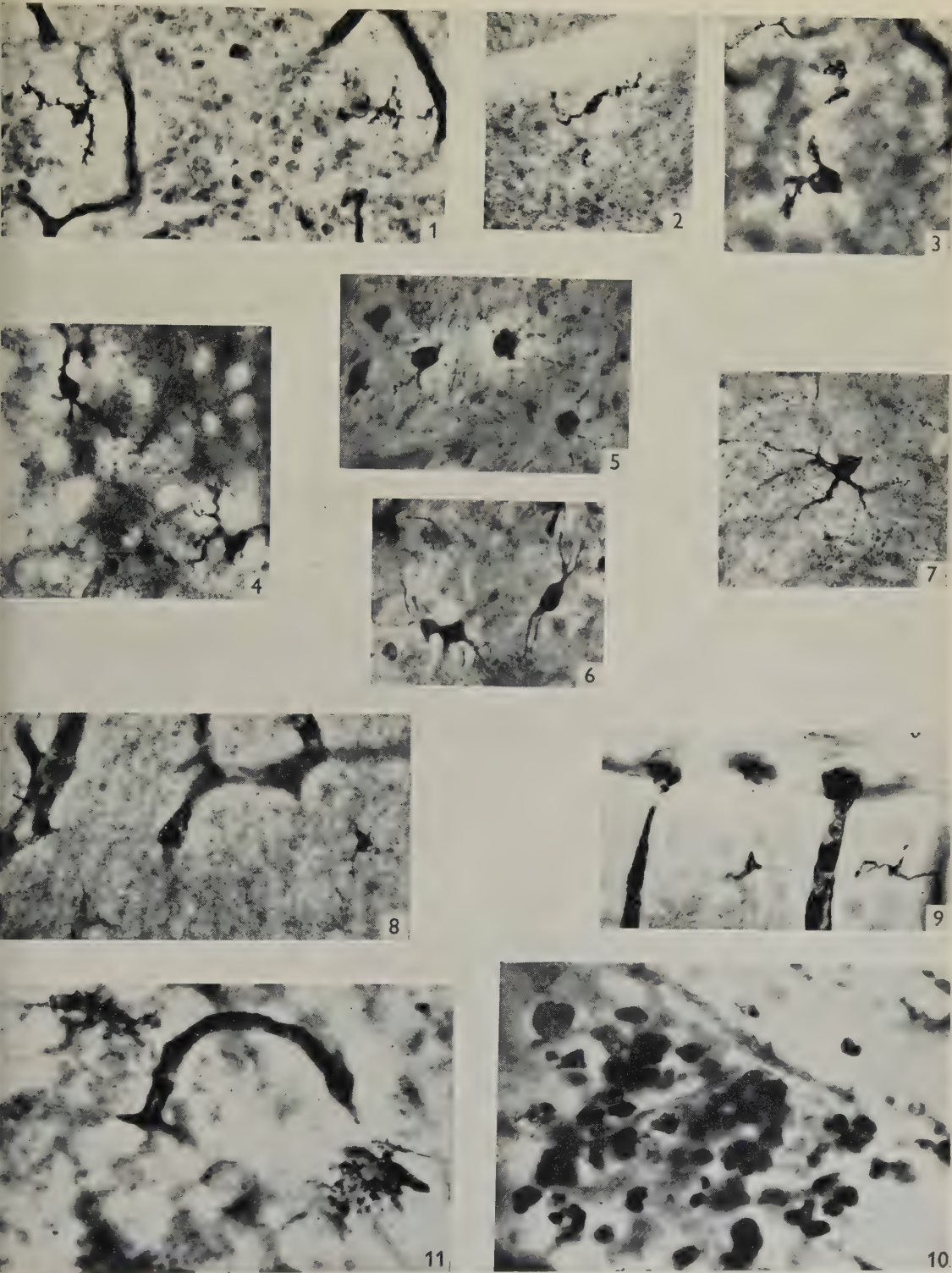
## PLATE 2

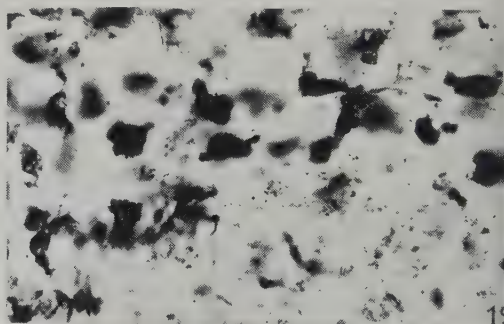
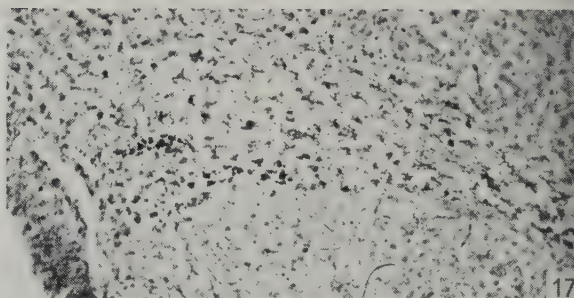
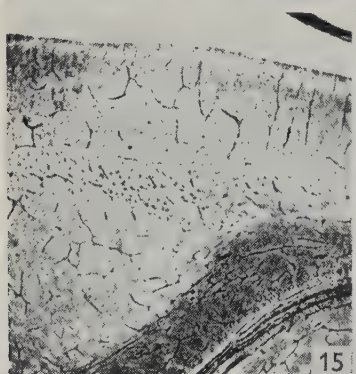
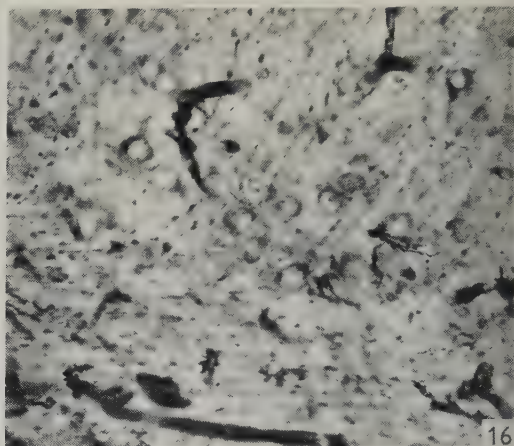
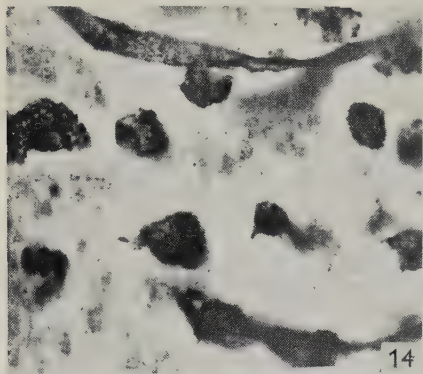
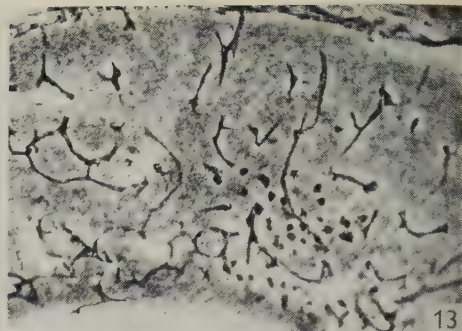
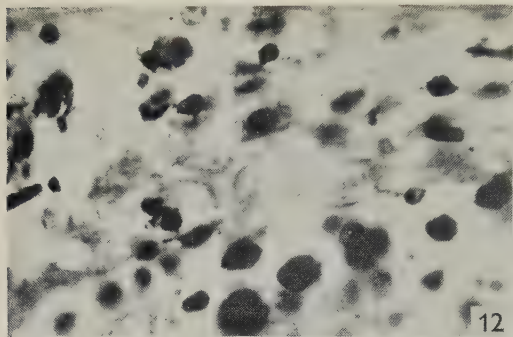
- Fig. 12. 17 mm. rat embryo. Amoeboid phagocytic cells in plane between spinal muscles, cervical region. Hortega,  $\times 325$ .
- Fig. 13. 19 mm. rat embryo, cerebral vesicle. Note accumulation of amoeboid cells. Hortega,  $\times 75$ .
- Fig. 14. High-power view of fig. 13. Note character of amoeboid cells, granular cytoplasm with few short ragged processes. Hortega,  $\times 465$ .
- Fig. 15. 25 mm. rat embryo, cerebral vesicle. Note definite layer of amoeboid type cells in the thickness of the hemisphere wall. Hortega,  $\times 38$ .
- Fig. 16. Seven-day-old rat, deepest part of cerebral cortex. Note finely beaded fibres turning up from the callosal radiation into the cortex. Microglia is scattered along the course of these fibres. Hortega,  $\times 270$ .
- Fig. 17. Twelve-day-old rat. Well-marked Hortega 'fountain' of immature microglia. Lateral ventricle to the left.  $\times 110$ .
- Fig. 18. Twelve-day-old rat treated with 11.25 mg. cortisone during 3rd, 4th, and 5th days after birth. Note suppression of Hortega 'fountain'. Lateral ventricle to the left.  $\times 110$ .
- Fig. 19. High-power view of fig. 17. Note amoeboid and immature character of microglia.  $\times 325$ .

## PLATE 3

- Fig. 20. High-power view of fig. 18. The few microglial cells present are branched and mature in type. Compound granular corpuscle type of cell is absent.  $\times 325$ .
- Fig. 21. 5.5 cm. human foetus, cervical cord. Note isolated bipolar type of microglia immediately beneath the ependyma of the central canal which is to the right. No obvious origin of such cells can be made out.  $\times 440$ .
- Fig. 22. 5.5 cm. human embryo, medulla oblongata. Note isolated branched cells of large size in midline raphe. Smaller microglial cells are present elsewhere in the section.  $\times 105$ .
- Fig. 23. High-power view of fig. 22.  $\times 325$ .
- Fig. 24. 5.5 cm. human embryo. Granular amoeboid cells beneath ependyma of midbrain.  $\times 260$ .
- Fig. 25. 7.0 cm. human embryo, internal capsule. Islands of microglia present.  $\times 57$ .
- Fig. 26. High-power view of fig. 25, showing character of the microglia. Thick granular processes contain tiny vesicles.  $\times 325$ .
- Fig. 27. 15.5 cm. human embryo. Cerebral cortex of frontal region. Island of deeply argyrophilic cells mingled with fibres is shown.  $\times 54$ .
- Fig. 28. Same specimen as fig. 27. Frontal cortex showing a group of three microglial cells which are on the fringe of an island like that shown in fig. 27.  $\times 400$ .
- Fig. 29. 2.6 cm. sheep embryo, basal ganglia. Lateral ventricle above and to the left. Isolated large coarsely branched microglia in upper part of field, some close up against the ependyma. Rounded amoeboid cells in lower part of field.  $\times 110$ .
- Fig. 30. High-power view of a well-branched cell present in fig. 29.  $\times 450$ .

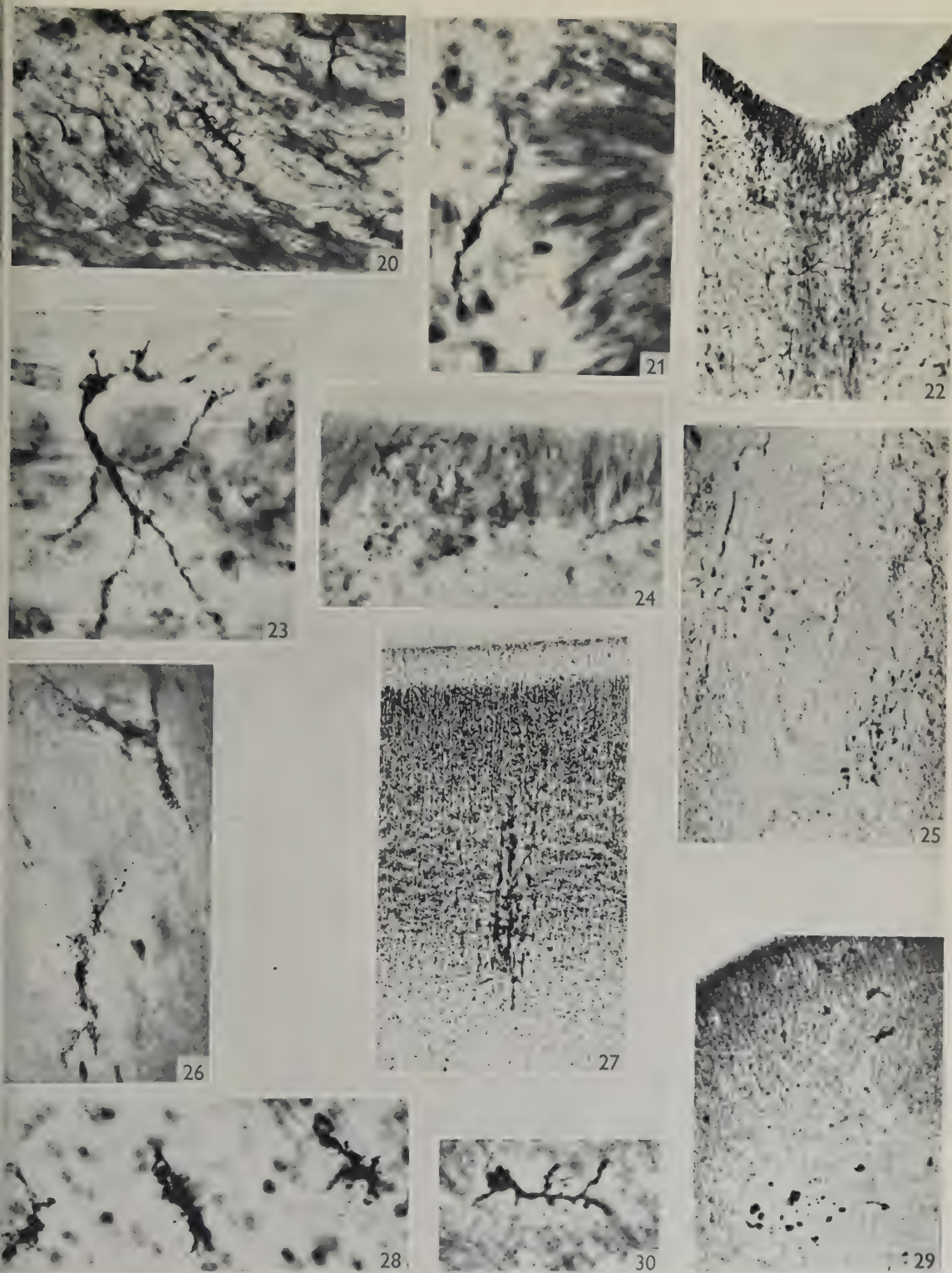






FIELD—OBSERVATIONS ON THE DEVELOPMENT OF MICROGLIA









# THE ARTERIAL SUPPLY OF THE HUMAN PROSTATE AND SEMINAL VESICLES

By E. J. CLEGG

*Department of Anatomy, University of Liverpool*

## INTRODUCTION

Much of the difficulty experienced in evaluating and comparing work on vascular anatomy is a result of lack of uniformity in nomenclature, and the literature on this particular region of the arterial system is no exception.

Previous workers appear to be divided on the question of the existence of a specific artery to the prostate gland (Kraas, 1935; Awataguti, 1939), but the measure of agreement is greater than would appear from a superficial survey of the literature, and differences in results may be explained as resulting from differences in terminology, rather than from fundamental anatomical variations.

The object of this report is to describe in some detail the blood supply to the human prostate and seminal vesicles, to compare the various systems of nomenclature used by previous workers, and to provide an accurate representation of the vascular patterns as a basis for surgical practice.

## MATERIALS AND METHODS

Fresh post-mortem material was used whenever possible, the ages of the subjects varying between 36 and 64 years. In no case did the prostate show pathological hypertrophy.

After ligating the common and external iliac arteries close to their origin the former was cannulated distally, and an injection of 10–15 ml. of a radio-opaque medium ('Micropaque'; Damancy and Co. Ltd) was made. This injection mass combines high density for radiographic purposes with good colour contrast for dissection. The injection was made at pressures varying from 100 to 200 mm. mercury; it was found that at lower pressures the viscosity of the medium prevented good filling of the vessels.

When a sufficient quantity of medium had been introduced (this could be judged by the appearance of the vessels on the superior surface of the bladder and the pubic branches of the obturator artery), the cannula was removed, and the pelvis eviscerated. A certain amount of leakage occurred when the parietal branches of the internal iliac artery were divided, but this loss was not serious.

Subsequent, a polythene cannula was tied in the superior rectal artery above its bifurcation, and the specimen radiographed with the rectum nearest the plate, and the emptied bladder drawn upwards. Without moving the specimen, 5 ml. of 'Micropaque' were injected into the superior rectal artery, and a further radiograph was taken. Dissection was commenced as soon as possible, the specimens being fixed in formol saline, and the dissected specimen radiographed again in certain cases.

Clearing by the Spalteholtz method was not employed because of the difficulties inherent in such large specimens.

A total of twenty-one pelvic halves were examined, of which four were pelvic halves from dissecting-room specimens. Of the remaining seventeen, seven were whole pelvic contents in which one side only (the right) had been injected, and the remaining ten comprised five bilaterally injected specimens. In the case of the superior rectal artery, the injections were bilateral. Hence, in this respect, a total of twenty-eight pelvic halves were examined.

The anastomoses between vessels of opposite sides were studied radiologically and by dissection in six of the seven unilaterally injected specimens.

In the study of the general topography of the vascular patterns, more reliance was placed on dissection than on the radiographical findings. The latter method was used to indicate the adequacy of the injection and to assist the analysis of the arterial supply of the organs by dissection.

## RESULTS

Despite the anatomical contiguity of the organs, the blood vessels supplying the prostate gland and seminal vesicle have less in common than might be expected. The two organs will, therefore, be considered separately.

### *The arterial supply of the prostate gland*

In all cases the gland is supplied by the prostatic branch of the prostatic-vesical artery, which is always a well-defined trunk of variable origin (Table 1). As a rule, the vessels of the two sides are of comparable size, but in one case the left prostatic artery was much larger than the right, and crossed the midline on the anterior surface of the gland.

Table 1. *The origin of the prostatic-vesical artery (Text-fig. 1)*

Gluteo-pudendal trunk	Origin of umbilical artery	Umbilical artery	Common trunk with vesiculo-deferential artery	Internal pudendal artery	Obturator artery
9	2	2	2	1	1

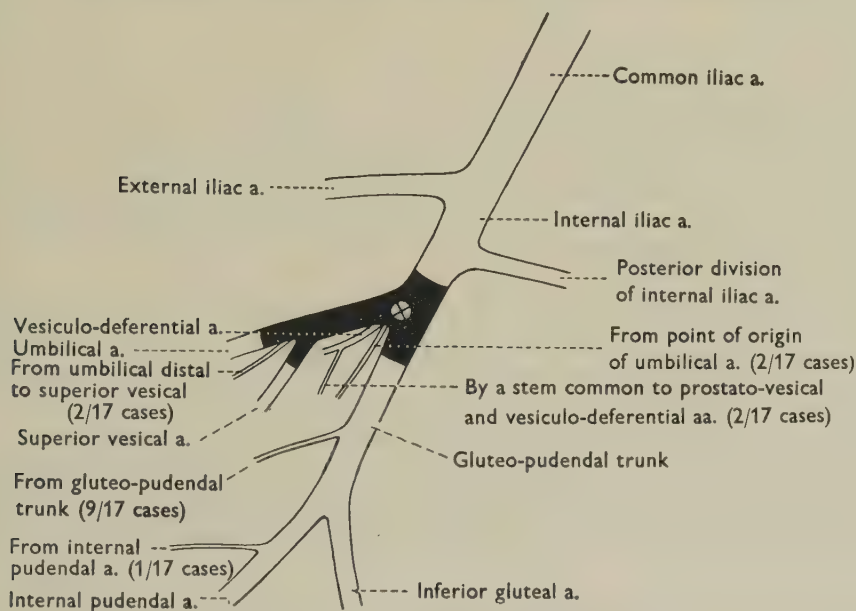
The course of the prostatic-vesical artery is fairly constant. It passes obliquely downwards, forwards and medially on the antero-inferior surface of the bladder towards the prostate gland, at a varying distance from which it divides into its two terminal branches, the inferior vesical and prostatic arteries (Pl. 1, fig. 1). This subdivision is by no means constant, however, as is shown in Table 2.

Table 2. *Mode of division of the prostatic-vesical artery*

Prostatic and inferior vesical arteries	Large prostatic artery—small inferior vesical artery	Several inferior vesical arteries	No inferior vesical artery
6	5	3	5



The prostatic artery, on the other hand, is a constant branch of this trunk. It reaches the gland on its antero-lateral surface, and passing down the lateral border (Pl. 1, figs. 2, 3), gives off fine twigs to the surface of the organ (Table 3), finally terminating as a bunch of small vessels which supply the pelvic floor. Some of these twigs pass to the rectum and anal canal, and may be classed as middle rectal arteries. Not infrequently one of these vessels may be enlarged, and in such a case it may appear that the prostatic artery is a branch of the middle rectal.



Text-fig. 1. Diagrammatic representation of the sites from which the prostatic artery can arise. The shaded area shows the region from which the vesiculo-deferential artery may arise and X marks its most frequent site of origin.

Table 3. *Distribution of the prostatic artery to the gland*

Branches to both surfaces	Anterior branches larger than posterior	Anterior surface only	Posterior surface only
18	8	6	2*

\* In these cases (two sides of one pelvis) the artery passed on a more posterior plane than usual to supply only the posterior surface of the gland. In this pelvis the anterior branches of the artery were replaced by two large vessels which descended across the pelvic floor from the obturator artery.

Another not infrequent branch of the prostatic artery is the *posterior vesicular artery* (author's terminology). This vessel, which, as its name suggests, supplies the posterior aspect of the seminal vesicle, arose from the prostatic artery in eight out of fourteen cases, and in one case it had a common origin with the latter from the gluteo-pudendal trunk.

The superior rectal artery was found to supply the gland in nine out of twenty-

eight cases, this total being made up of three pelves in which the supply was bilateral, and three in which the left branch only supplied the gland.

These vessels are given off from their parent trunk opposite the middle third of the rectum, and pass around its lateral borders, eventually running deeply below the peritoneal floor of the recto-vesical pouch to the upper lateral angle of the gland, where they communicate with the other vessels of supply, some of these communications being of considerable size.

Other arteries which occasionally supplied the gland included the posterior vesicular (three cases) and vesiculo-deferential arteries (three cases), but since their main territory of supply is the seminal vesicle, they will be described in the next section.

It is interesting to note here that in the three cases in which the vesiculo-deferential artery supplied the prostate, the prostatic artery supplied the anterior surface of the gland only, and a similar finding was recorded in two out of the three cases in which the posterior vesicular artery supplied the gland. When a supply was derived from the superior rectal artery, the posterior branches of the prostatic artery were smaller than usual in all but one case.

A very prominent feature of the vessels on the surface of the prostate is their tortuosity (Pl. 2, fig. 1). This is of a characteristic 'corkscrew' pattern, and can be seen in vessels penetrating the stroma of the gland (Pl. 2, fig. 2). It is not so well marked on the surface of the seminal vesicle.

A study of six unilaterally injected specimens revealed that there were few anastomoses of arteriolar size or larger between vessels of opposite sides in the gland itself. This could be equally well demonstrated in radiographs of bilaterally injected specimens (Pl. 1, figs. 2, 3), in which very few connexions could be seen between the vessels of the two sides.

#### *The arterial supply of the seminal vesicle*

Just as the prostatic artery is the main source of supply of the prostate gland, so the vesiculo-deferential artery (Pl. 1, fig. 2) supplies the seminal vesicle in all cases examined.

The various sites of origin of this vessel are shown in Table 4.

Table 4. *The origin of the vesiculo-deferential artery*

At the site of origin of the umbilical artery from the internal iliac artery	First cm. of umbilical artery	Superior vesical artery	Umbilical artery distal to superior vesical artery	Anterior division of internal iliac proximal to umbilical artery	Gluteo-pudendal trunk
10	2	2	1	1	1

In one case also the vessel arose as two separate stems, a vasal artery and a vesicular artery arising with the superior vesical artery. The two cases in which the vesiculo-deferential and prostatovesical arteries arose by a common trunk have been grouped here with the most frequent mode of origin of the vesiculo-deferential artery.

The vessel constantly passes medially behind the supero-lateral border of the bladder, in front of the ureter, to which it gives branches (Cerf, 1895; Farabeuf, 1905; Braithwaite, 1952). It continues medially to the lateral end of the seminal vesicle where it normally divides into three branches, vesical, deferential and *anterior vesicular* (author's terminology). Not infrequently the artery gives off its deferential branches between the ureter and seminal vesicle, occasionally lateral to the ureter. The vesical branch gives off the marginal trigonal artery (Braithwaite, 1952), and a less constant branch which runs towards the opposite side along the upper boundary of the trigone.

The anterior vesicular artery is the largest branch of the vesiculo-deferential artery. In nine out of thirteen cases it was double, in three single, and in one case there were four arteries. If single it usually divides into two or more branches which ramify in the depths of the grooves on the anterior surface of the seminal vesicle. In cases in which two arteries are present, the upper one usually anastomoses over the upper border of the vesicle with the posterior vesicular artery, and along the lower border of the ampulla of the vas deferens with the proximal deferential artery.

The lower anterior vesicular artery runs along the front of the inferior border of the seminal vesicle. It may communicate with the vesical branches of the vesiculo-deferential artery.

The vasal artery generally arises as a single stem which runs to the vas deferens, and there divides into proximal and distal branches. In four cases out of fifteen, however, the distal deferential artery was given off first, and the segment of vas deferens between its origin and that of the proximal deferential artery presumably received its blood supply directly from the vesiculo-deferential artery, which lies closely applied to the vas in this position.

The *posterior vesicular artery* (author's terminology) supplies mainly the posterior surface of the seminal vesicle, although it invariably sends branches which pass over the upper border of the vesicle to anastomose with the upper anterior vesicular artery. Its origin is shown in Table 5.

Table 5. *The origin of the posterior vesicular artery*

Prostato-vesical artery	Gluteo-pudendal trunk	Vesiculo-deferential artery
8	5	1*

\* In this case the prostato-vesical and vesiculo-deferential arteries arose by a common stem which bifurcated after a course of 1 cm.

The artery is usually of small or moderate size, and passes medially upwards or downwards, according to its origin, to the lateral end of the seminal vesicle, where it turns downwards to run behind its inferior border, giving off several small branches which supply its posterior surface. Its anastomotic branches with the anterior vesicular artery have already been described.

As mentioned above, both the vesiculo-deferential and the posterior vesicular arteries supplied the prostate gland in three cases.



(a) In the three out of seventeen cases in which the former artery supplied the gland, one was via a large anastomosis with the superior rectal artery, in another the artery supplied the postero-lateral surface of the gland, and in the third, branches of the anterior vesicular artery passed downwards and forwards to supply the bladder neck and upper part of the prostate.

(b) In the three out of eighteen cases in which the posterior vesicular artery supplied the gland, it was by a continuation of the main stem of the vessel medially on the posterior surface of the organ. This stem was of small size and communicated with the other vessels to this region of the gland.

Occasional anastomoses between the two vesicles could be demonstrated, both by dissection and, with less certainty, by radiography. Quite often the vessels supplying the ampullae communicated across the midline posteriorly, and in two cases a fairly large vessel passed between the proximal vasal arteries on the posterior surface of the trigone. The vessels on the vesicles did not show the same degree of tortuosity as those on the surface of the prostate gland.

## DISCUSSION

### *The arterial supply of the prostate gland*

There would appear to be a difference of opinion in the literature concerning the existence of a definitive prostatic artery. In favour of its existence are Cerf (1895), Poirier (1896), Farabeuf (1905), Loeschke (1920), Adrion (1922), Tsaknis (1929) and Kraas (1935). Henle (1868) mentions, without giving a reference, an alternative name for the middle rectal artery, the '*arteria prostatica s. vesico-prostatica*'. The existence of a prostatic artery is specifically denied by Cammerat (1923), Flocks (1937) and Awataguti (1939). Testut (1895), without actually denying the existence of the vessel, emphasized the variability of the vascular pedicles to the gland.

This apparent difference of opinion is almost certainly due to a confusion in nomenclature. Those authors who deny the existence of a prostatic artery, emphasize the constancy of the supply of the gland from the middle rectal and inferior vesical arteries, either of which, from the results of the present investigation, may spring from the vessel which also supplies the prostate gland. Of these three vessels, by far the most constant is the prostatic artery, and the trunk which gives rise to this vessel and the inferior vesical and middle rectal arteries might well be termed the 'prostato-vesico-haemorrhoidal trunk'. However, the middle rectal vessel is so varied in its origin, that an equally accurate and less cumbersome term might be the 'prostato-vesical artery'. This interpretation has already been used by Cerf (1895), Poirier (1896) and Farabeuf (1905).

The site of origin of the prostato-vesical artery agrees fairly well with the work of Braithwaite (1952). This author found that the 'inferior vesical artery' arose from the gluteo-pudendal trunk or one of its branches in 59.1 % of cases, compared with ten out of seventeen (58.8 %) in the present series. The main difference lies in the different frequencies of origin of the vessel from the trunk or its individual branches.

The course of the prostatic artery, and its division into anterior and posterior branches follow the usual descriptions.

In the present series, a surprisingly high number of cases was found (32.1 %) in which the prostate received branches from the superior rectal artery. This mode of

supply has been described by Awataguti (1939), who found the superior rectal artery supplying the gland in 14.3% of cases, while Quénu (1893) stated that the superior rectal artery supplies the prostate by unilateral branches.

*The arterial supply of the seminal vesicle*

Little work appears to have been performed on the more detailed anatomy of the arterial circulation in this organ, and some authors, and most of the standard textbooks, even ignore the existence of the vesiculo-deferential artery. Probably the best descriptions of this vessel are by Cerf (1895), Delbet (1923) and Farabeuf (1905).

The site of origin of the vessel is reasonably constant, although the present findings differ slightly from those of Awataguti and Braithwaite. The former found the 'deferential artery' to arise from the umbilical artery in 90% of cases, and the latter in 93.2%, compared with 83.3% in the present series. However, there is agreement that the commonest site of origin is from the angle between the umbilical and gluteopudendal trunks.

The anterior and posterior vesicular arteries have not been hitherto described, although it is possible that the latter is the 'accessory middle rectal artery' of Awataguti, which supplies the prostate in ten out of seventy-seven pelvic halves examined, and arises in forty out of forty-two cases from the gluteo-pudendal trunk or one of its branches.

The tortuosity of the vessels supplying the prostate has received brief reference in the works of Poirier (1896), and Bumpus & Antopol (1934). This phenomenon is not so well marked in the seminal vesicles, but a definite 'corkscrew-like' appearance may be observed in the vasal artery during its course on the vas deferens. It was observed that the tortuosities are much more marked in the vessels on the posterior surface of the gland.

The reason for these appearances is unknown. Possibly they are related to variations in size or position, or to the amount of smooth muscle in the organs where they are marked. The fact that the vessels on the surface of the seminal vesicle, a much less muscular organ, do not show these tortuosities to the same extent, lends a certain amount of support to the last hypothesis.

SUMMARY

1. In a total of twenty-one pelvic halves, the blood supply to the prostate gland and seminal vesicle was studied by dissection and radiologically.

2. A definitive prostatic artery was found in all cases examined; it was the most constant branch of the prostatico-vesical artery.

3. The superior rectal artery was found to supply the gland in 32.1% of cases, a much higher figure than that of Awataguti (1939).

4. The vesiculo-deferential artery was found to supply the seminal vesicle in all cases through its anterior vesicular branch (author's terminology). In eight cases out of fifteen the posterior vesicular artery (author's terminology) was a branch of the prostatico-vesical artery, and in six cases a branch of the gluteo-pudendal trunk.

5. It is considered that variations in the nomenclature of blood vessels in this region account, in the main, for the wide diversity of findings in the blood supply of the prostate and seminal vesicles.

This work was suggested by Prof. R. G. Harrison, and I am indebted to him, and to Dr J. L. Braithwaite, for their constant advice and encouragement. The photographs were prepared by Messrs L. G. Cooper and C. FitzSimon, and the diagram was drawn by Mr D. J. Kidd and Miss G. O. Thomas.

The expenses of the research were in part defrayed by a grant from the Medical Research Council.

#### REFERENCES

- ADRION, W. (1922). Ein Beitrag zur Ätiologie der Prostatahypertrophie. *Beitr. path. Anat.* **70**, 179–202.
- AWATAGUTI, S. (1939). Beitrag zur Kenntnis der Arterienverteilung im männlichen Becken mit besonderer Berücksichtigung der Blutversorgung der Prostata. *Mitt. allg. Path. Sendai*, **10**, 58–92.
- BRAITHWAITE, J. L. (1952). M.D. Thesis, University of Manchester.
- BUMPUS, H. C. & ANTROPOL, W. (1934). Distribution of blood to prostatic urethra; demonstration. *J. Urol.* **32**, 354–358.
- CAMMERATT, R. (1923). Zur Frage der Prostatahypertrophie. *Virchows Arch.* **245**, 27–42.
- CERF, L. (1895). Thèse de Paris.
- DELBET, P. (1923). In P. Poirier and A. Charpy, *Traité d'Anatomie Humaine*, Tome v, nouvelle ed. Paris: Masson et Cie.
- FARABEUF, L.-H. (1905). *Les vaisseaux sanguins des organes génito-urinaires du périnée et du pelvis. Amplification de la thèse du Dr Leon Cerf*. Paris: Masson et Cie.
- FLOCKS, R. H. (1937). The arterial distribution within the prostate gland; its role in transurethral prostatic resection. *J. Urol.* **37**, 524–548.
- HENLE, J. (1868). *Handbuch der systematischen Anatomie des Menschen*. Braunschweig: Vieweg and Sohn.
- KRAAS, E. (1935). Die arterielle Gefäßversorgung von Blasenhalsh und Prostata. *Arch. klin. Chir.* **183**, 595–606.
- LOESCHKE, (1920). Über Wesen und Entstehung der Prostatahypertrophie mit Demonstrationen. *Münch. med. Wschr.* **68**, 302.
- POIRIER, P. (1896). In Poirier's *Traité d'Anatomie Humaine*. Paris: Masson et Cie.
- QUÉNU, M. (1893). Des artères du rectum et de l'anus chez l'homme et chez la femme. *Bull. Soc. anat. Paris*, 5me série, Tom. VII, 703–708.
- TESTUT, L. (1895). *Traité d'Anatomie Humaine*, Tom. III. Paris.
- TSAKNIS, D. (1929). Thèse de Paris.

#### EXPLANATION OF PLATES

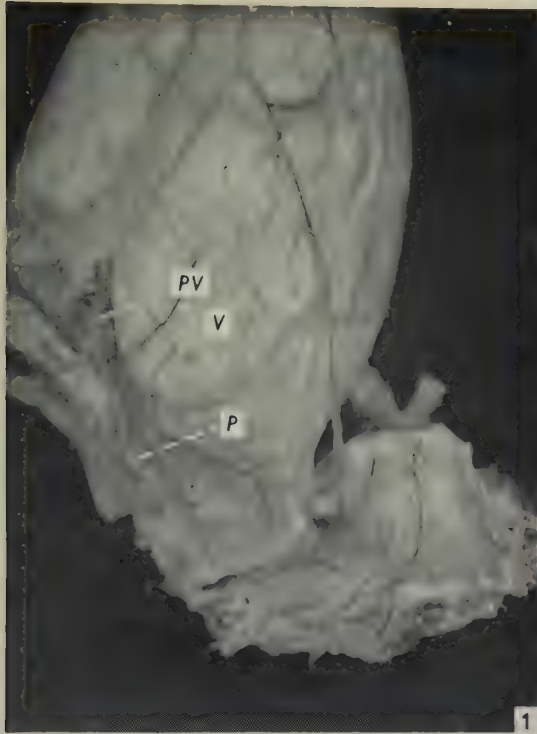
##### PLATE 1

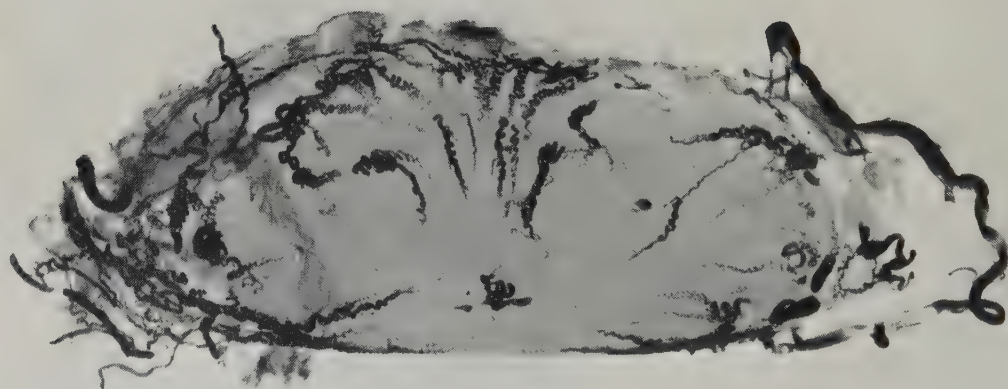
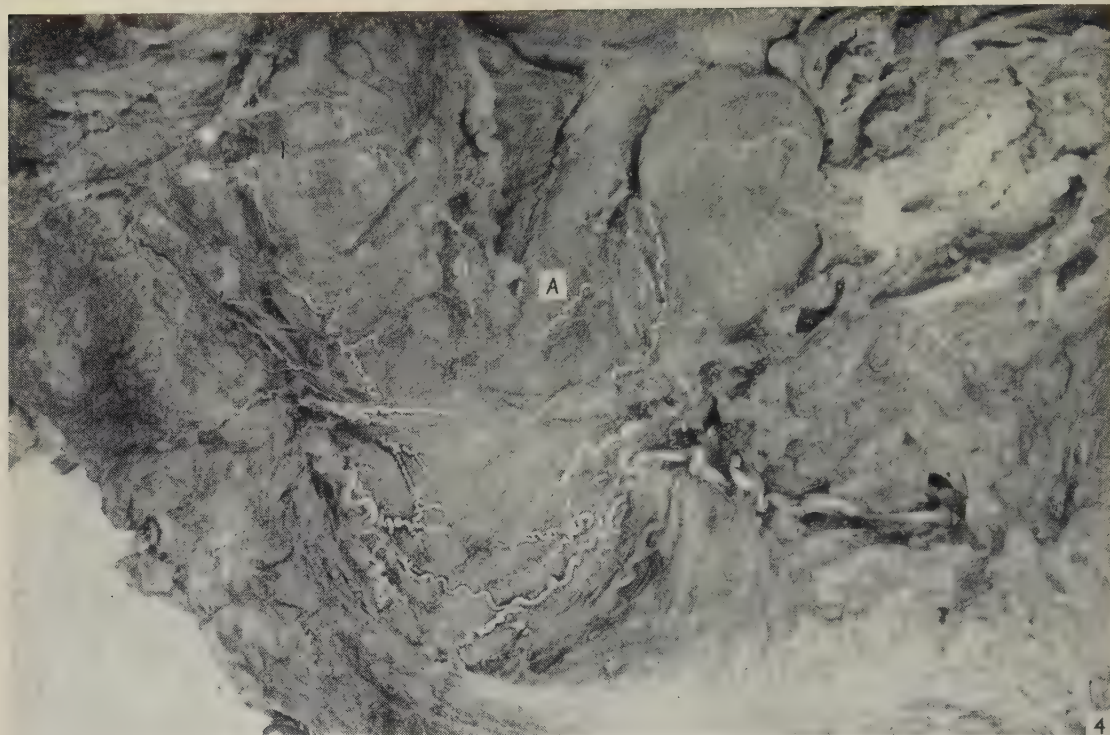
- Fig. 1. Photograph of the bladder and prostate of a stillborn foetus, injected with indian ink. The division of the prostato-vesical artery (PV) into prostatic (P) and inferior vesical branches (V) can be seen. In this case the inferior vesical artery was small, and the prostatic artery supplied a large vessel running between the bladder and prostate, giving branches to both. ( $\times 1.5$ ). (Courtesy of Dr J. L. Braithwaite.)
- Fig. 2. Radiograph of the bladder and prostate of a man aged 36, unilaterally injected with 'Micropaque'. The prostatic artery (P) can be seen coursing along the lateral border of the gland, finally terminating by supplying the anal canal. The vesiculo-deferential artery (VD) can be seen to be a branch of the superior vesical artery.
- Fig. 3. Radiograph of the prostate (P) and seminal vesicle (V) of a man aged 47, unilaterally injected with 'Micropaque'. The bladder has been removed. The ramifications of the vessels on the surfaces of the seminal vesicle are visible. The 'corkscrew-like' tortuosities of the prostatic vessels are easily distinguished from those of the vesicular arteries.

##### PLATE 2

- Fig. 4. Photograph of the arteries on the posterior surface of the prostate of a 47-year-old man after bilateral injection with 'Micropaque'. The tortuosity of the vessels is evident. A vessel on the ampulla (A) of the vas deferens shows similar features.
- Fig. 5. Radiograph of a 1 cm. thick transverse section of the prostate illustrated in fig. 4. The typical tortuosity of the vessels penetrating the substance of the gland can be seen.







# OBSERVATIONS ON THE VALVES OF HOUSTON IN THE HUMAN EMBRYO AND FOETUS

BY P. H. S. SILVER

*Department of Anatomy, Middlesex Hospital Medical School, London, W. 1*

Valve-like 'projections' interrupting the lumen of the rectum were first described by Houston in 1830, but a study of these structures in the human embryo and foetus has never been undertaken specifically. The growth of the hindgut and urorectal septum, and the breakdown of the cloacal membrane in the embryo are common knowledge, but the subsequent appearance of the valves of Houston after the embryonic stages are completed has only been touched on by Baur (1863), Johnson (1914) and Cunningham (1951). None of these authors describes the histological features of the valves either during their earlier development or, in the foetus, once they are fully formed. The findings of Baur and Johnson are summarized in Table 1. This lack of knowledge in the foetus is matched by the confusion and contradictions which are to be found in the literature describing these structures in the adult.

Table 1. *Summary of Baur (1863) and Johnson's (1914) findings*

Author	Age of embryo	Description of Houston's valve
Baur (1863)	3rd month	'2 cross folds, right and left at same level, 3 cm. from anal verge'*
Johnson (1914)	12 weeks, 70 mm. C.R. 14 weeks, 99 mm. C.R. 19 weeks, 140 mm. C.R.	Nil '2 small folds in lower ampulla' '3 folds obliquely placed,' 22-20 18-15 and 13-10 mm. from anal verge
Baur (1863)	4-5 months	3 valves: R, strongly marked. L, two small folds
	4-5 months	1 valve: R, 1.1 cm. from anal verge
Johnson (1914)	24 weeks, 187 mm. C.R.	L, R, R, 30, 25, 20 mm. from anal verge. L valve was largest
	Stillborn (size not stated):	
	(1)	3 valves: R, R, L, 50, 40, and 30 mm. from anal verge
	(2)	2 valves: R, L, 45 and 35 mm. from anal verge
	(3)	2 valves: L, R, 40 and 30 mm. from anal verge
	(4)	Valves absent: rectum distended with meconium

\* The figure of 3 cm. must be due to a misprint in Baur's paper.

Much of this confusion may be traced back to Houston's original paper. He gave a detailed description of the valves in the following words: 'the position of the largest and most regular valve is about three inches from the anus opposite the base of the bladder. The fold of next most frequent existence is placed at the upper end of the rectum. The third in order occupies a point about midway between these, and the fourth, or that most rarely present is attached to the side of the gut, about an inch above the anus.' In another paragraph he describes these valves as lying



respectively on the right, on the left, anteriorly, and in the left posterior position. Thus when only three valves are present (the 'average number' according to Houston) they would be expected to lie on the right, on the left and anteriorly. Surprisingly the figure which illustrates Houston's paper shows them lying on the left, on the right, and on the left. In short, the text and the figure do not agree with each other.

Houston's words have been paraphrased in certain modern accounts, see Gray (1949), Buchanan (1949), Morgan (1936), Miles (1944). But in 1830 'the rectum' was said to stretch from the region of the upper piece of the sacrum to the anus and was divided for descriptive purposes into three parts. The old first part of the rectum, with its 'meso-rectum' is to-day included in the sigmoid colon; the old second part is still called the rectum, and the third part is now called the anal canal. When Houston described a valve on the right, 'at the upper end of the rectum', he meant a structure in what is now called the sigmoid colon; and when Gray in 1860 described this valve 'at the commencement of the rectum', he accurately conveyed Houston's meaning at that time. But when these same words occur in modern accounts of Houston's valves, the reader cannot be sure whether they should be interpreted in the sense in which Houston used them, or in the light of the revised terminology.

Quain (1914), Testut (1905), Cunningham (1951) and Gabriel (1948) describe three valves interrupting the rectal lumen. Their accounts agree with Houston's figure, but differ from his verbal description in two respects: (i) the main valve, which Houston placed on the anterior aspect of the rectum, they place on the right or right anterior aspect of the bowel, (ii) it is the uppermost valve which is omitted and not the lowermost, although according to Houston the lowermost is the most infrequent. On the other hand, Wood Jones (1911) and Buchanan (1949) describe the uppermost valve as constant and the second and fourth as 'inconstant puckerings of the mucous membrane'.

Hyrtl (1853) called Houston's main valve 'sphincter ani tertius'. Kohlrausch (1854) disagreed with this name on the grounds that the fold did not, as a rule, contain fibres of the circular muscle coat, and suggested instead the name 'plica transversalis recti'. Nélaton (1859) described a circular fold of mucous membrane with a central foramen (une espèce de diaphragme) as the most usual appearance, but the foramen could shift in any direction and produce a crescentic fold attached to any of the four walls of the rectum. Vance (1878) supported Houston's description of the valves forming a spiral attachment to the rectal wall, like the intestine of the 'serpent or dog fish'.

Although in the adult the position of the valves is associated with the sinuous course of the rectum, this association has not been demonstrated in development. In the child the rectum is straighter than in the adult, but the valves are well developed nevertheless (Daniels, 1937). Quain (1914), Buchanan (1949) and Cunningham (1951) attribute the valves to the thickened anterior and posterior parts of the longitudinal muscle coat, which, by shortening, produce, or at least maintain, infolding of the lateral rectal wall.

Wood Jones (1911) regarded Houston's main valve as of fundamental morphological significance, demarcating the junction zone between hindgut and cloaca.

In describing the constituent layers of the valves many authors (Quain, 1914;

Gray, 1949; Cunningham, 1951; Buchanan, 1949) follow Houston, who showed by dissection 'a duplication of the mucous membrane enclosing between its laminae some cellular tissue with a few circular muscle fibres'. Bacon (1949) follows this description in his text, but in his illustration (fig. 54) he shows the full thickness of the circular muscle coat to be included. He specifically mentions the absence of the longitudinal coat. Gabriel (1948), however, includes all the layers of the rectal wall within the valves of Houston. Pennington (1900) emphasized the extreme variability of structure from a fold simply composed of mucous membrane, to one containing all the layers of the rectal wall.

Baur (1863) looked for Houston's valves in the wolf, bear, wombat, *Semnopethicus maurus*, *Lemur tardigradus*, *Cebus opellus*, *Jemina troglodytes* and the rhinoceros. In none of these animals did he find any such valves. He believed them to be peculiar features of the human rectum.

From the foregoing brief review of the literature, it will be appreciated that many problems remain unsolved, and that many divergent accounts have yet to be reconciled with each other in the adult. This developmental study was undertaken to see whether a more definite picture would emerge in the embryo and foetus.

#### MATERIAL AND METHODS

Details of the twenty-one human embryos and foetuses used in this investigation are summarized in Table 2.

After fixation the embryos were sectioned *in toto* and stained with haematoxylin and eosin. In the foetuses, after fixation, the rectum was removed and photographed, but was left attached either to the sacrum or to the anterior pelvic structures so as to prevent distortion occurring during the preliminary stages of histological manipulation.

#### Results

Pl. 1, fig. 1, shows a sagittal section of the 21 mm. embryo. Although the front of the sacrum is flat, the rectum is curved with a backward convexity and its lumen has two terminal dilatations, the upper one of which is well shown in the figure. In the wall of the rectum the muscle coats have already begun to differentiate and can be seen as condensations of mesenchyme.

The 55 mm. embryo (Pl. 1, fig. 2) shows the earliest appearance of a valve of Houston. The longitudinal and circular muscle coats are clearly seen. The submucous tissue is markedly thickened on the right anterior aspect of the rectum, 1.9 mm. from the anal verge, and 0.7 mm. below the recto-vesical pouch. The muscularis mucosae is demarcated as a condensation of mesenchyme cells, but it is no more evident in this region than in the adjacent parts of the rectal wall. The epithelium, projecting into the lumen in the form of *villous-like processes*, consists of a single layer of columnar cells.

The region of thickened submucous tissue is invaded by fibres of the circular muscle coat, but not, as yet, by the outer longitudinal fibres. Large blood vessels penetrate the gut wall in this region.

The rectum, at this stage, is far in advance of the rest of the large intestine in its histological development.

Table 2

Crown rump length (mm.)	Plane of section	Orientation pattern of valves from above downwards	Distance of valves from anal verge (mm.)	Distance of pouch of Douglas to anal verge (mm.)
13.5	Sagittal	None	—	0.4
21	Sagittal	None	—	1.0
30	Cross	None	—	—
55	Sagittal	RA	1.9	2.6
66	Sagittal	—	—	3
76	Coronal	R	8.5	—
		L	7	—
		R	6	6.5
90	Coronal	R	6.5	—
		L	5	—
95	Coronal	R	10.5	—
		R	9.5	—
		L	8.25	—
		R	6.75	7
		L	5	—
104	Sagittal	L	9	—
		R	7	8
		L	5.5	—
110	Sagittal	L	11	—
		R	7.5	9
		L	6	—
120	Sagittal	L	7.25	8.25
140	Coronal	L	13	—
		R	10	10
		L	8	—
160	Coronal	R	18	—
		L	14	—
		R	10	—
170	Naked eye	L	14	—
		R	12	11.25
170	Coronal	R	26	—
		R	24	—
		L	16.5	—
		R	12	11.5
		L	8.75	—
180	Coronal	R	17.5	—
		L	13.5	14.5
		R	10	—
180	Coronal	L	20	—
		R	14.5	15.5
		La	11.5	—
190	Coronal	L	20.5	—
		R	16.5	16.5
		L	12	—
270	Coronal	L	32	—
		R	22	20
		L	15	—
300	Naked eye	L	24	—
		R	20	22
300+	Naked eye	R	37	—
		L	28	30



The 76 mm. embryo shows the appearance of three valves. The middle valve is on the left 7 mm. from the anal verge, whereas the other valves lie on the right at distances of 8.5 and 6 mm. respectively. The Pouch of Douglas is 6.5 mm. from the verge. The middle valve is visible by the naked eye, as an indentation of the outer wall of the rectum. Histological section shows that the longitudinal muscle coat is incorporated into the valve at this stage, but the muscularis mucosae shows no special development in the region of Houston's valves.

The 95 mm. foetus shows five similar indentations on the outer rectal wall, but they do not interrupt the general direction of the rectum which is still lying in the sagittal plane. The upper two valves are not so well developed as the lower three (Pl. 1, fig. 3), and there is no suggestion of the valves being arranged in spiral fashion.

In all the later stages examined Houston's valves were invariably demonstrated, but in one case (120 mm.), in which the rectum had been distended with blood-stained meconium, the valves are very inconspicuous.

In two cases the rectum is noticeably tortuous when seen from in front, e.g. Pl. 1, fig. 4*a, b*, the indentations which produced the valves actually overlapping each other. In other cases the tortuosity is not so conspicuous as in this specimen, but the anterior and posterior parts of the longitudinal muscle coats pursue a sinuous course down the front and back of the rectum (Pl. 1, fig. 5).

Pl. 1, fig. 6, shows the only example in which there is a suggestion of a spiral arrangement of the rectal valves. The histological sections, however, show that the spiral fold consists only of mucous membrane, and therefore cannot be regarded as part of the Houston's valve system (see discussion below for histological criteria of Houston's valves in the foetus). Pl. 1, fig. 7, shows a single case (180 mm.) in which a valve is seen projecting from the anterior as well as from the side wall.

Pl. 1, fig. 8, shows the histological findings, in the second 180 mm. specimen. After fixation when viewed from without it had the appearance of a simple, almost straight tube, and the presence of valves was not suspected. After clearing, the wall of the rectum was translucent, but the lumen could be seen since it was stained with meconium. There were three deep indentations, one above and one below on the right, and one on the left, in the intermediate position. Pl. 1, fig. 8, shows the histological appearances, which clearly demonstrate that all the layers of the rectal wall are included in the valves. Bridging the clefts in the lateral walls the connective tissue can be discerned, which on naked eye examination gave to the rectum its deceptive appearance of straightness.

#### DISCUSSION

The great variations in the histological features of Houston's valves in the adult rectum have been emphasized by Pennington (1900). In the foetus the picture is more consistent, and it is possible to lay down as a definite criterion that the valve should contain all the layers of the rectal wall including the outer longitudinal muscle coat. This criterion, however, does not apply at the tips of the crescentic valves.

The valves are produced by indentation of the rectal wall, first by the submucosa then by the circular and finally by the longitudinal muscle coat; they are not

produced as a result of the sacculations which are a feature of the adult rectum. Neither can the valves be said to be produced or maintained by the shortening of the anterior and posterior parts of the longitudinal muscle coats since, in many specimens, these fibres can be seen to pursue a sinuous course down the front or back of the rectum. If the contraction of these fibres had produced the infolding of the lateral rectal wall, then they would be expected to lie on a straight line.

Table 3. *Summary of orientation patterns of Houston's valves*

Orientation pattern: from above downwards	<div style="display: flex; align-items: center; justify-content: space-around;"> <div style="font-size: 4em; margin-right: 10px;">{</div> <div style="text-align: center;"> <div style="margin-bottom: 5px;">L   R   —   R   L   L   R   L</div> <div style="margin-bottom: 5px;">R   L   R   L   L   L   L   —</div> </div> <div style="margin-left: 10px;"> <div style="text-align: center; margin-bottom: 5px;">R R</div> <div style="text-align: center;">L   L   R   L</div> </div> </div>									
Number	6	3	2	2	1	2				

Note: It is not clear from the measurements (see Table 2) whether the specimen with a single valve on the left, should be regarded as a variant of the [L, R, L] pattern or not.

Nor is the muscularis mucosae specialized or precociously developed in the region of the valves, and there is no evidence that it has initiated the projection of the sub-mucosa; in brief it must be concluded that the valves are produced by a process of differential growth.

The orientation pattern of Houston's verbal description did not occur in this series (see Table 3 for summary). An explanation for this is suggested. It may be that in the adult, owing to the backward sacral curvature of the rectum, and, lower down, owing to the forward angulation produced by the contraction of the pubo-rectalis, that creases appear on the front and back of the bowel which become continuous with the folds which are already present at the sides. Consequently, the right valve comes to lie in the right anterior position as described by Gabriel, or to be completely incorporated into the anterior crease; the lowest valve, in the same way, comes to lie in the left posterior position. The posterior and anterior curvatures, which are well-marked features of the adult rectum, are much less conspicuous in the foetus.

There seems to be no support for the thesis that the main valve has any fundamental morphological significance, since the first appearance of the valves is long after the junction of gut and cloaca, and in any case such a theory leaves the presence of more than one valve unexplained. This theory, moreover, would give rise to the expectation that the valves should be demonstrable in many related mammalian species.

Lastly, the question of the function of Houston's valves needs to be considered. In the human foetus, the capacity of the rectum when distended with meconium, seems to be increased by the obliteration of the valves. But this cannot be the sole explanation for their presence since the same functional needs must arise in related species in which the valves have not been found (Baur, 1863). It may be that the valves are associated with the adoption of the upright posture. Even if this is so, we still know nothing definite about their function.

Clearly these problems require further study by the methods of comparative anatomy, not only in the adult but also in the embryo and foetus.

## SUMMARY

1. A review of the literature concerning Houston's valves in the adult has shown that there is disagreement on many material anatomical points. This is true regarding (a) position and number of valves, and (b) degree to which the layers of the rectal wall are incorporated into the valves. As far as can be discovered, the valves have never previously been studied specifically in the embryo and foetus.

2. Observations have been made in twenty-one human embryos and foetuses. The valves make their appearance towards the end of the third month: the submucosa becomes thickened and is invaded successively by the circular and longitudinal muscle coats and finally by the perirectal connective tissue. This is a consistent and reliable picture. The anterior and posterior parts of the longitudinal muscle coats play no part either in the production or maintenance of the valves.

3. When seen from outside, the rectum usually appears straight or almost straight, but this belies the nature of the lumen which zigzags past the valves of Houston, because they project from the lateral walls like horizontal baffle plates. When the rectum is distended with meconium the valves are taken up into the rectal wall.

I wish to thank Prof. E. W. Walls for his constant encouragement and interest, Prof. J. Kirk and Mr O. V. Lloyd-Davies with whom I was fortunate in being able to discuss various problems which arose in the course of this work, Mr P. A. Runnicles for most of the histology and photography, Mr K. L. Frampton who provided invaluable assistance from time to time, and the Central Research Fund of the University of London for the purchase of a microtome.

## REFERENCES

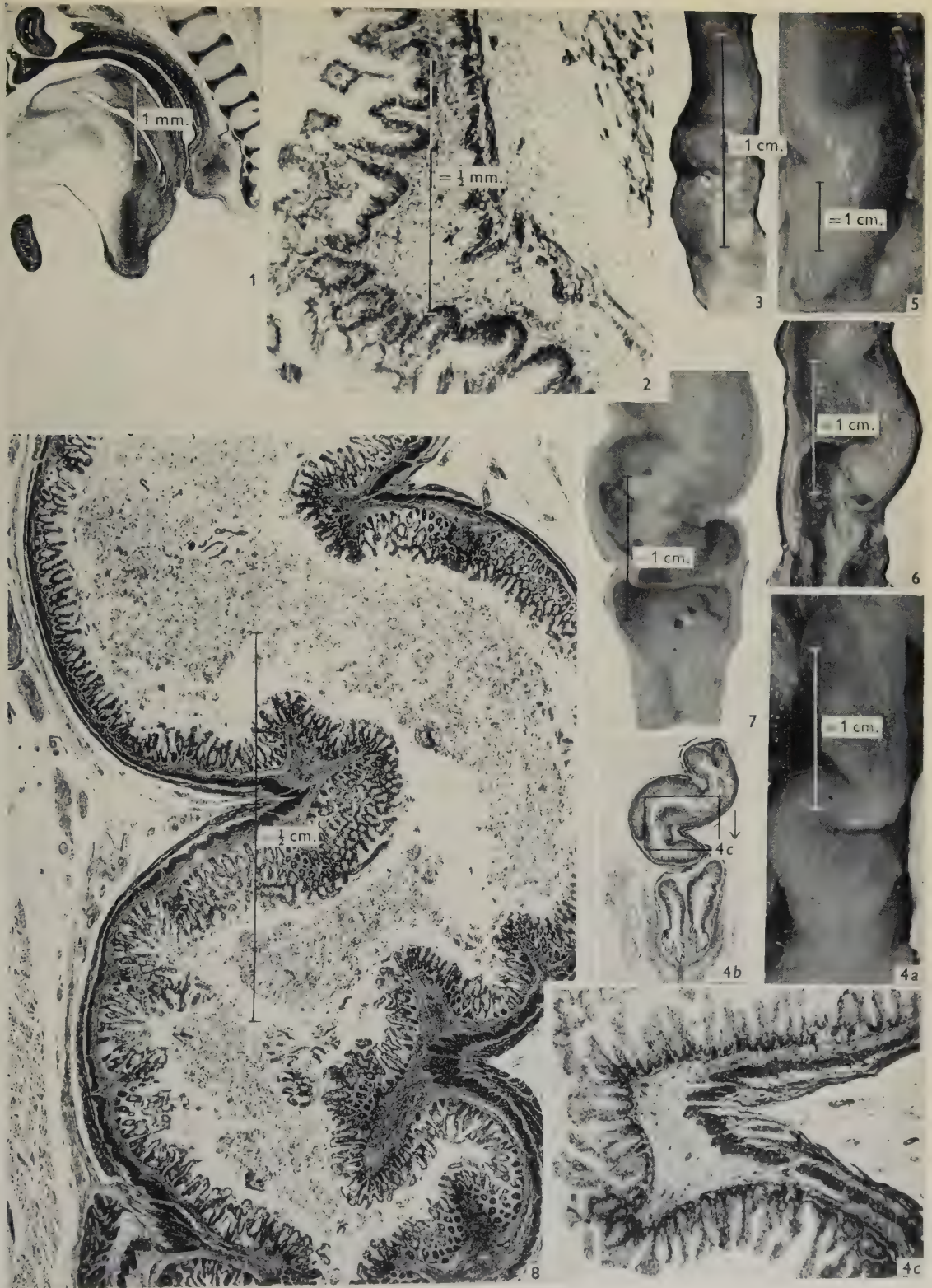
- BACON, H. E. (1949). *Anus, Rectum, Sigmoid Colon. Diagnosis and Treatment*, 3rd ed. Philadelphia: Lippincott.
- BAUR, H. (1863). Ueber die Falten des Mastdarms. *Beitr. Anat. Physiol.* 3, 1-38.
- BUCHANAN, A. M. (1949). *Manual of Anatomy*, 8th ed. by F. W. Jones. London: Baillière, Tindall and Cox.
- CUNNINGHAM, D. J. (1951). *Textbook of Anatomy*, 9th ed. by J. C. Brash. London: Oxford University Press.
- DANIELS, E. A. (1937). Rectal disorders in childhood. *Amer. J. Dis. Child.* 54, 573-589.
- GABRIEL, W. B. (1948). *The Principles and Practice of Rectal Surgery*, 4th ed. London: H. K. Lewis.
- GRAY, H. (1860). *Anatomy, Descriptive and Applied*, 2nd ed. London: John W. Parker.
- GRAY, H. (1949). *Anatomy, Descriptive and Applied*, 30th ed. by T. B. Johnston and J. Whillis. London: Longmans, Green and Co.
- HOUSTON, J. (1830). Observations on the mucous membrane of the rectum. *Dublin Hosp. Rep.* 5, 158-165.
- HYRTL, J. (1853). *Handbuch der Topographischen Anatomie*. Wilhelm Braumüller.
- JOHNSON, F. P. (1914). The development of the rectum in the human embryo. *Amer. J. Anat.* 16, 1-59.
- JONES, F. W. (1911). The delimitation of the rectum and its subdivisions. *Proc. R. Soc. Med.* 4, pt. 3 (Surgical section), 85-98.
- KOHLRAUSCH, O. (1854). *Zur Anatomie und Physiologie der Beckenorgane*. Leipzig: Hirzel.
- MILES, W. E. (1944). *Rectal Surgery*. London: Cassell.
- MORGAN, C. N. (1936). Surgical anatomy of the anal canal and rectum. *Post. Grad. med. J.* 12, 287-314.



- NÉLATON, A. (1859). *Eléments de Pathologie chirurgicale*. Paris: Germer, Baillière.
- PENNINGTON, J. R. (1900). New points in the anatomy and histology of the rectum and colon. *J. Amer. med. Ass.* **35**, 1520-1526.
- QUAIN, J. (1914). *Elements of Anatomy. Splanchnology*, 2, pt. 2. London: Longmans, Green and Co.
- TESTUT, L. (1905). *Traité d'anatomie humaine*, 4, 5me éd. Paris: Doin.
- VANCE, R. A. (1878). Rudimentary structures in the rectum, spiral folds and valvular projections of its mucous membrane. *Med. Surg. Rep. Philad.* **38**, 203-205.

## EXPLANATION OF PLATE

- Fig. 1. Sagittal section through the pelvis of 21 mm. embryo, to show the position and shape of the rectum.
- Fig. 2. Parasagittal section through the rectum of 55 mm. embryo, to show the early appearance of a valve of Houston. The mucous membrane is hypertrophied, and fibres of the circular muscle coat are invading the submucosa.
- Fig. 3. Photograph of the rectum of a 95 mm. embryo seen from *in front*. The delicate sleeve of connective tissue surrounding the bowel is not thick enough to conceal the indentations of the lateral walls by the developing valves. Orientation pattern R, R, L, R, L.
- Fig. 4. Naked eye appearance (*a*), and two histological sections (*b* and *c*) of the rectum of 140 mm. embryo. (*a*) The rectum is seen *from behind*. Orientation pattern L, R, L. Notice the extreme tortuosity. (*b*) Coronal section to show the general appearance of the rectum, and emphasizing more clearly the features which are seen in (*a*). (*c*) An enlargement of the intermediate valve, seen in fig. 4*b* for comparison with figure 2. Notice the blending of some of the longitudinal fibres with the circular fibres near the apex of the valve.
- Fig. 5. Rectum of 270 mm. foetus seen from *in front*. Notice the sinuous course of the anterior longitudinal fibres.
- Fig. 6. Rectum of 190 mm. foetus. The specimen has been sectioned in the coronal plane, and the figure shows the *posterior half seen from in front*. Orientation pattern L, R, L. Notice the fold of mucous membrane running obliquely upwards from the lowest valve towards the intermediate valve.
- Fig. 7. Rectum of 180 mm. foetus. The specimen was sectioned in the coronal plane, and the figure shows the *anterior half from behind*. Notice the fold continuous across the anterior wall from the lowermost valve.
- Fig. 8. Rectum of 180 mm. foetus. Coronal section seen *from behind*. Orientation pattern R, L, R. All layers of rectal wall are incorporated into Houston's valves.







## SOME FACTORS INFLUENCING ANGULATION OF THE NECK OF THE MAMMALIAN TALUS

By C. H. BARNETT

*Department of Anatomy, St Thomas's Hospital Medical School*

### INTRODUCTION

The degree of angulation of the talar neck varies greatly from one mammalian species to another, and it would be expected that this diversity has either a functional or a taxonomic significance. From his exhaustive study of the mammalian talus, Volkov (1904) concluded that the angle of the neck has a 'quite exceptional importance' in assessing the functions of the foot, while Duckworth (1904) has stated that 'this character distinguishes the Hominidae clearly from the Simiidae...'.

In an attempt to determine the reason for this structural variation, a number of tali have been examined, particular attention being paid to the different factors that could have influenced the angle of the neck during the course of evolution.

### MATERIAL AND METHODS

Ninety-eight adult mammalian limbs have been studied, including twenty-five wet specimens. In addition, thirty-five human feet have been examined from twenty cadavers and fifteen post-mortem subjects.

The angle of the neck of the talus is commonly determined by laying the disarticulated bone upon a horizontal plane and measuring the angle between the long axis of the neck and that of the trochlear surface, as seen from above. Although this is a convenient method (Inkster, 1927), it has two serious defects. In many animals the trochlear margins are not straight and the neck is short with irregularly shaped sides. Consistent figures are then very difficult to obtain. An even more serious source of error, as Appleton (1913) emphasized, is that the orientation of the talus in the living foot is seldom identical with that of the excised bone placed on a horizontal surface. Since the neck is usually inclined downward as well as medially, slight changes in the orientation of the bone may cause apparent alterations in the talar angle. For this reason, measurements of the angle have been recorded mainly in wet specimens or in dried osteoligamentous preparations of the whole foot.

A large talar angle could be produced either by medial deviation of the neck within the foot or by obliquity of the trochlear margins. The latter might be caused by obliquity of the trochlear margins only, the upper surface of the talus forming part of a screw (Fig. 1), or alternatively by deviation of the whole trochlear surface, with a consequent obliquity of the axis of rotation at the ankle joint. These three theoretical possibilities are shown in Fig. 2.

To assess which of these factors was responsible for a large angle of the neck in any specimen, the long axis of the foot was first determined by joining the middle of the forefoot—or, in pentadactyl limbs, the middle digit—to the mid-point of the heel.

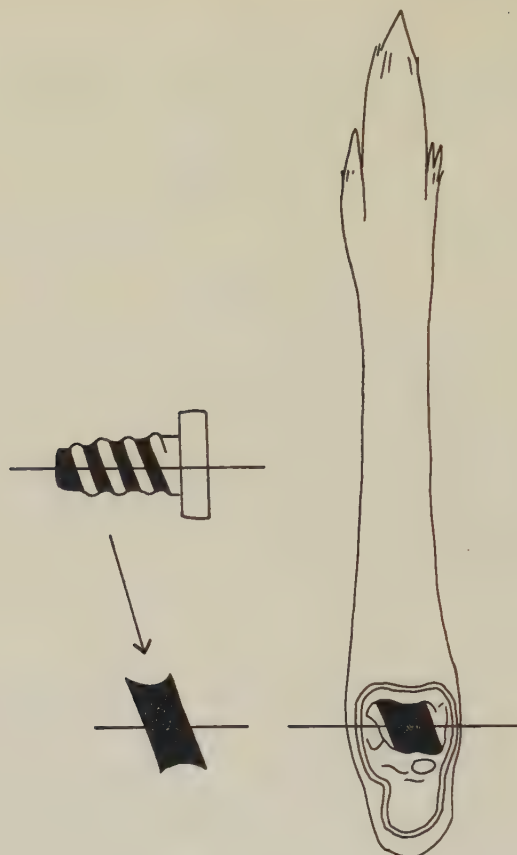


Fig. 1. Left foot of wallaby. The trochlea is part of a right-handed screw set transversely in the foot.

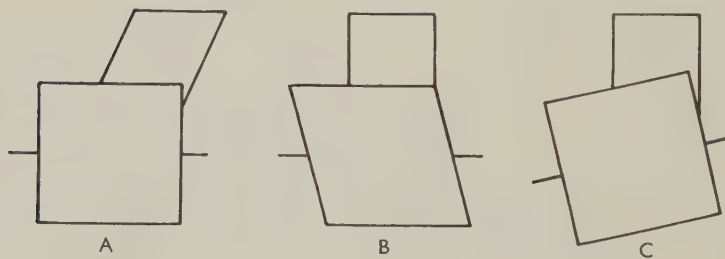


Fig. 2. Three factors affecting the angle of the talar neck: A: medial deviation of the neck relative to the long axis of the foot; B: obliquity of the trochlear margins relative to the long axis of the foot; C: deviation of the trochlear surface, and the axis of rotation at the ankle joint, relative to the long axis of the foot.

The direction of the long axis of the talar neck, the plane of the trochlear margins and the position of the axis of rotation at the ankle joint were then determined relative to the long axis of the foot. The ankle joint axis was deduced from the contours of the medial and lateral profiles of the talus as previously described (Barnett & Napier, 1952, 1953). So far as possible all the tarsal joints were maintained in the neutral position during the measurements.

#### OBSERVATIONS AND DISCUSSION

The simplest type of talus is one in which the long axis of the neck and both trochlear margins are parallel to the long axis of the foot, which is itself at right angles to the axis of rotation at the ankle joint. This type, with no angulation of the neck, is found characteristically in species with a long narrow foot adapted for a cursorial type of gait, such as the rabbit (*Oryctolagus cuniculus*), rabbit bandicoot (*Thalacomys lagotis*), tiger-cat (*Dasyurus maculatus*), Tasmanian devil (*Sarcophilus harrisi*) and many of the cursorial artiodactyla.

Table 1 indicates the relative importance of the three factors—medial deviation of the neck, obliquity of the trochlear margins, and deviation of the whole trochlea with a corresponding obliquity of the axis of rotation at the ankle joint—in forty species with a large talar angle.

##### *Medial deviation of the neck*

This is an important factor in increasing the talar angle in two types of animals. Firstly, it occurs in those in which the force transmitted down the leg is deviated within the foot towards its medial side, as in arboreal forms with a prehensile hallux. Secondly, it is present in animals with a wide foot in which the head of the talus lies to the medial side of the calcaneum, a relationship of the two proximal tarsal bones which is commonly found in animals with a burrowing habit or a plantigrade gait (Table 1).

##### *Obliquity of the trochlear margins*

In those species in which the trochlear surface forms part of a screw, there may be considerable lateral movement of the talus upon the tibia during flexion and extension at the ankle joint, especially if the obliquity of the trochlear margins and the total range of ankle movement are both large. It can be shown by a modification of Manter's method (1941) that in the horse, for example, the talus moves bodily about 4 mm. in a lateral direction as it passes from full dorsiflexion to full plantarflexion. A screw type of articulation is very stable, being especially well suited to resist antero-posterior strains. While some of the animals with this type of talus are heavy, active creatures with elongated limbs, capable of jumping, they do not all possess ankle joints that palpably require especial protection against such strains. Moreover, the actual pitch of the screw varies greatly even in those creatures that conform to this pattern; consequently the angle of the neck may differ in related species (e.g. lion, tiger) due solely to differences in the pitch of the screw of which the trochlea is part.



Table 1. *The factors responsible for a large talar angle in forty species*

Species	No. of specimens examined	Type of specimen	Total angle of talar neck	Medial deviation of neck	Obliquity of trochlear margins	Deviation of ankle axis
<i>Castor fiber</i> (beaver)	2	O.L.	15	15	0	0
<i>Centetes caudatus</i> (tenrec)	2	O.L.	12	12	0	0
<i>Tarsius spectrum</i> (tarsier)	3	W.	30	30	0	0
<i>Chiromys madagascariensis</i> (aye-aye)	1	O.L.	15	15	0	0
<i>Hapale jacchus</i> (marmoset)	2	W.	12	12	0	0
<i>Mandrillus sphinx</i> (mandrill)	3	B.	30	30	0	0
<i>Procavia capensis</i> (Cape coney)	3	W.	25	25	0	0
<i>Mustela erminea</i> (stoat)	1	W.	10	10	0	0
<i>Panthera leo</i> (lion)	3	B.	20	20	0	0
<i>Tapirus indicus</i> (tapir)	2	B.	20	0	20	0
<i>Equus caballus</i> (horse)	3	B.	20	0	20	0
<i>Pedetes cafer</i> (jumping hare)	1	O.L.	15	0	15	0
<i>Macropus rufogriseus</i> (wallaby)	2	W.	20	0	20	0
<i>Dendrolagus matschei</i> (tree-kangaroo)	1	W.	15	0	15	0
<i>Potorous tridactylus</i> (rat kangaroo)	1	W.	20	0	20	0
<i>Dendrohyrax dorsalis</i> (tree-coney)	3	O.L.	30	15	3	12
<i>Hydrochoerus hydrochoeris</i> (capybara)	2	B.	25	10	15	0
<i>Hystrix cristata</i> (porcupine)	3	W.	20	10	0	10
<i>Chrysochloris asiatica</i> (golden mole)	2	W.	15	10	0	5
<i>Erinaceus europaeus</i> (hedgehog)	3	W.	10	5	0	5
<i>Bathyergus maritimus</i> (sand-mole)	2	O.L.	20	5	0	15
<i>Canis familiaris</i> (dog)	5	W.	15	12	3	0
<i>Ailuropoda melanoleuca</i> (giant panda)	2	B.	25	2	15	8
<i>Meles meles</i> (badger)	2	W.	25	10	5	0
<i>Priodontes gigas</i> (giant armadillo)	2	B.	10	10	5	-5
<i>Ateles frontatus</i> (spider monkey)	2	W.	25	20	0	5
<i>Cercopithecus collaris</i> (mangabey)	1	W.	30	25	5	0
<i>Mystax ursulus</i> (tamarin)	1	O.L.	25	20	0	5
<i>Macaca mulatta</i> (rhesus monkey)	4	W.	25	20	0	5
<i>Hylobates hoolock</i> (gibbon)	2	O.L.	35	25	0	10
<i>Papio papio</i> (guinea baboon)	3	W.	25	15	5	5
<i>Simia satyrus</i> (orang utan)	3	B.	30	18	8	4
<i>Gorilla gorilla</i> (gorilla)	3	B.	28	20	3	5
<i>Homo sapiens</i> (man)	35	W.	18	15	-5	8
<i>Tamandua tetradactyla</i> (lesser ant-eater)	2	O.L.	20	10	0	10
<i>Panthera tigris</i> (tiger)	3	B.	25	20	5	0
<i>Myrmecophaga jubata</i> (giant ant-eater)	2	B.	20	30	0	-10
<i>Felis domestica</i> (cat)	4	W.	18	13	5	0
<i>Myocaster coypus</i> (coypu)	2	O.L.	15	12	3	0
<i>Orycteropus afer</i> (aardvark)	2	W.	10	15	0	-5

O.L.: osteoligamentous preparations studied; W.: at least 1 wet specimen studied also; B.: only dried bones studied. In the first fifteen species listed, only one factor is responsible for the large talar angle; in the remainder at least two factors play a part.

#### *Deviation of the ankle axis*

In most species the axis of rotation at the ankle joint is transverse, i.e., at right angles to the long axis of the foot. In certain arboreal forms the ankle axis is oblique, usually lying approximately parallel to the line joining the heads of the lateral four metatarsal bones. In man also, it has been shown by modifications of Manter's method (Manter, 1941; Barnett, 1953; Hicks, 1953) that the ankle axis is not usually at right angles to the long axis of the foot as is often stated (e.g., Elftman & Manter, 1935; Wood Jones, 1949), but is directed backwards and laterally, so that a line at right angles to it makes an angle of 5 to 10 degrees with the long axis of the foot. This lateral deviation of the trochlea in man is partly masked by a *medially* directed obliquity of the trochlear margins relative to the ankle axis (Table 1). Thus the

human left talus may be regarded as part of a left-handed screw the long axis of which points backwards and laterally\* (Fig. 3).

The obliquity of the human ankle axis is in large measure the cause of the supination of the foot that accompanies plantarflexion; this rotational movement is only slightly reduced in patients whose subtalar and midtarsal joints have been arthrodeseised.

Some fossorial animals exhibit a similar deviation of the ankle axis from the transverse position, leading to marked supination—or, in certain instances, pronation—of the foot during plantarflexion. This rotational movement is probably of value in burrowing.

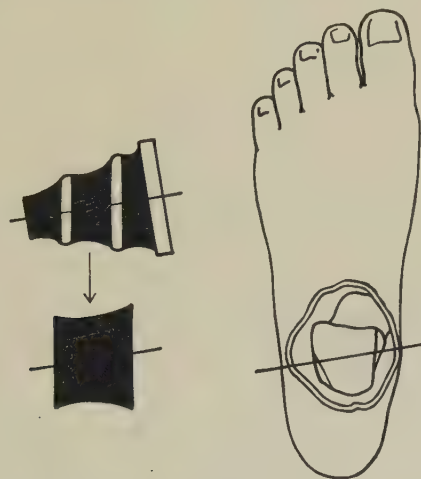


Fig. 3. Left foot (human). The trochlea is part of a left-handed screw set obliquely in the foot (compare Fig. 1).

### CONCLUSIONS

Since several distinct factors have influenced the angle of the talar neck during the course of evolution, the magnitude of the angle in any species is in itself useless as a guide to function unless the relative importance of these factors has been analysed. A very small talar angle is typical of cursorial forms. Medial deviation of the neck within the foot is found in species with a wide foot, for example, those with a plantigrade gait or a fossorial habit, and in arboreal species in which the body weight is deviated within the foot towards the medial side. A talus in which the trochlear margins are obliquely set within the foot is seen characteristically but not exclusively in heavy animals capable of jumping. A foot in which the whole trochlea, and thus the axis of rotation at the ankle joint, is deviated with respect to the long axis of the foot is found in some arboreal primates, and also in man and certain fossorial species.

As the extensive studies of Matthew (1937) make clear, the tarsal bones are not uncommonly preserved intact in fossil form, and it may be that a reassessment of fossil tali along the lines suggested would throw some light on the habits of extinct fauna.

\* The analogy is admittedly imperfect: the axis of rotation at the human ankle is not, in fact, stationary.

## SUMMARY

1. Ninety-eight mammalian tali, representing forty-eight species, have been examined with respect to the degree of angulation of the neck.

2. Estimates of the talar angle may be misleading, not only because of fallacies and difficulties in the technique commonly adopted for its measurement, but because three distinct factors may be responsible for a large angle.

3. In forty species with a large talar angle, the relative importance of these factors is listed, and the functional significance of each of the three is discussed.

I am indebted to Prof. D. V. Davies, the late Prof. F. Wood Jones, Dr F. C. Fraser and Miss J. E. King for helpful advice and for the provision of material.

## REFERENCES

- APPLETON, A. B. (1913). Note on a variable feature of the astragalus. *J. Anat., Lond.*, **47**, 123-142.
- BARNETT, C. H. (1953). Further observations upon the axis of rotation at the human ankle joint. *Proc. Anat. Soc. G.B.I.*, in *J. Anat., Lond.*, **87**, 449.
- BARNETT, C. H. & NAPIER, J. R. (1952). The axis of rotation at the ankle joint. *J. Anat., Lond.*, **86**, 1-9.
- BARNETT, C. H. & NAPIER, J. R. (1953). The rotatory mobility of the fibula in eutherian mammals. *J. Anat., Lond.*, **87**, 11-21.
- DUCKWORTH, W. L. H. (1904). *Morphology and Anthropology*. Cambridge University Press.
- ELFTMAN, H. & MANTER, J. (1935). The evolution of the human foot, with special reference to the joints. *J. Anat., Lond.*, **70**, 56-67.
- HICKS, J. H. (1953). The mechanics of the foot: I. The joints. *J. Anat., Lond.*, **87**, 345-357.
- INKSTER, R. G. (1927). The form of the talus. Thesis. Edinburgh University.
- JONES, F. WOOD (1949). *Structure and Function as seen in the Foot*, 2nd ed. London: Baillière, Tindall and Cox.
- MANTER, J. T. (1941). Movements of the subtalar and transverse tarsal joints. *Anat. Rec.* **80**, 397-410.
- MATTHEW, W. D. (1937). Paleocene faunas of the San Juan Basin, New Mexico. *Trans. Amer. phil. Soc.*, N.S., **30**.
- VOLKOV, T. (1904). Variations squelettiques du pied chez les primates et dans les races humaines. *Bull. Soc. Anthropol. Paris*, Ser. 5, **4**, 632-708.



## EXCISION AND REIMPLANTATION OF THE EPIPHYSEAL CARTILAGE OF THE RABBIT

BY P. A. RING

*Charing Cross Hospital Medical School*

The postnatal growth of a typical long bone is associated with activity within the epiphyseal plates, and within the articular cartilage which surmounts the ends of the bone. The early enlargement of the epiphysis takes place at the expense of both epiphyseal and articular cartilage, as the illustrations of Gottesleben (1939) clearly show. Later enlargement of the epiphysis occurs solely on its articular aspect (Boerema, 1942). The epiphyseal cartilage thus shows an early bipolarity, although its contribution to shaft elongation is always greater than its contribution to epiphyseal enlargement. With the formation of a terminal plate of bone upon its epiphyseal aspect, the epiphyseal cartilage loses this bipolarity, and its activities are restricted to new bone formation in the metaphyseal region. Payton (1933) has suggested that the region of this terminal plate is the site of absorption of epiphyseal bone. His deductions, however, from the madder-fed pig are circuitous and unsupported by the radiological evidence of Siegling (1941). The growth of the epiphysis itself has been fully discussed by Lacroix (1951).

In the present investigation the polarity of the epiphyseal cartilage has been investigated by experiments in which the plate has been excised and replaced in its normal site, either orientated normally, or rotated through 180° to bring the diaphyseal surface into contact with the epiphysis. The further growth of the bone, the extent, and the nature of the contribution from each end have been studied.

### METHOD

Rabbits aged from 2 to 5 weeks were used. Each animal was anaesthetized with ether, and the bones of the forelimb approached by a longitudinal incision. The distal end of the ulna was identified, and exposed for most of its circumference by freeing and retracting the adjacent tendons. The epiphyseal cartilage was isolated by transverse cuts passing through the immediately adjacent metaphyseal and epiphyseal bone, and this growth cartilage, together with its perichondrium, and a thin sliver of adjacent bone was removed. The excised portion was then replaced, either in its normal position, or reversed. With the latter procedure, the metaphyseal aspect of the cartilage with its adjacent bone lay against the epiphysis, and the epiphyseal aspect against the diaphysis. The tendons were allowed to fall together to hold the fragment in position, and the skin was sutured. No immobilization was necessary.

Each animal was radiographed after the operation and thereafter at weekly, and later fortnightly intervals. Animals were removed from the mother at 6 weeks and subsequently kept in a large wire run. They received a normal mixed diet supple-

mented by pellet feeding. At varying periods up to 28 weeks biopsies were performed. The animals were killed with Nembutal and the limbs radiographed. The bones of the forelimb were fixed in formol saline or Susa, and later decalcified. Following decalcification the distal ends of the radius and ulna were removed and sectioned.

All radiographs were taken with the animal in the prone position, each limb flexed at the elbow and the paw outstretched to give a standard dorso-ventral view of the distal part of the limb, and a lateral view of the proximal part. The tube-film distance was constant at 100 cm.; it can be demonstrated that at this distance the error due to magnification is less than 0.5 mm. and is equal on both sides. Each ulna was measured from its most proximal point to the centre of the distal articular surface to give its total length. The length of the distal epiphysis was measured from the centre of the translucency representing the epiphyseal plate to the centre of the articular cartilage. This measurement, when the epiphyseal plate is broad in the young animal, is about 1 mm. greater than that of the bony epiphysis.

### OBSERVATIONS

#### (a) *Excision and reimplantation in normal position*

The postoperative growth of the sixteen animals in this series is recorded in Table 1. The radiographs of thirteen of these animals show fusion of the metaphysis and epiphysis with the bony edges of the reimplanted epiphyseal cartilage within the

Table 1. *Excision and reimplantation of distal ulnar epiphyseal cartilage*

No.	Survival (weeks)	Growth (mm.)		Shortening (mm.)
		Operated	Control	
195	1	1	1	0
198	1½	3	3	0
197	2	4	4	0
219	2	6	7	1
220	2	5.5	7.5	2
196	3	5	6	1
296	3	8	9.5	1.5
222	5	10	10	0
143	7	22	24	2
104	8	13	26	13
90	9	12.5	27	14.5
140	11	28.5	29	0.5
142	11	26	27	1
84	19	20.5	32.5	12
141	24	39.5	40	0.5
75	28	36	41.5	5.5

first 14 days. Subsequent growth was, on the whole, undisturbed, shortening in the operated limb being less than 2 mm. in twelve animals and totalling 5.5 mm. in the thirteenth. The slight shortening occasionally observed was mainly apparent only towards the end of the period of growth.

The three animals in which little or no postoperative growth occurred merit consideration in detail. Failure of growth in rabbit 104 was associated with absorption of the graft followed by bony union between epiphysis and diaphysis. The graft in rabbit 90 at first appeared to grow in a normal manner, but subsequently flakes of calcified tissue appeared within the epiphyseal cartilage and premature epiphy-

seal fusion followed. The early radiographs of rabbit 84 showed the graft in position, and normal growth occurring. After 5 weeks, however, cross-union was apparent between the ulnar epiphysis and the shaft of the radius, probably due to damage of the periosteum of the latter during the operation. As a result of this cross-union further growth of the ulna resulted first in a dislocation of the radius, and secondly in a dislocation of the ulna at the elbow (Text-fig. 1). The strain to which the epiphyseal cartilage was subjected was probably responsible for its subsequent fusion.

Examination of the histological sections showed that the epiphyseal cartilage remained intact. The bone of the ulnar epiphysis and diaphysis united with the thin plates of bone on either side of the reimplanted cartilage within the first 2 post-operative weeks. The site of the union was for some time marked by an irregularity in the lamellae of the bone. The epiphyseal cartilage was at first considerably longer than normal, mainly due to an increase in length of the cartilage columns. This change was most marked during the third postoperative week. Sections from animals killed after a longer interval showed that the cartilage returned to its normal proportions, but in most animals showed a pronounced irregularity in the arrangement of its columns. In the centre of the epiphyseal cartilage an irregularity and enlargement of the cells of the reserve zone was often apparent, and was often associated with bone invasion from the epiphysis (Pl. 1, fig. 1).

Sections were available from two of the three animals which failed to grow. Rabbit 104 showed no trace of an epiphyseal cartilage. Sections of rabbit 90 showed a thin quiescent epiphyseal plate surrounded by irregular deposits of bone.



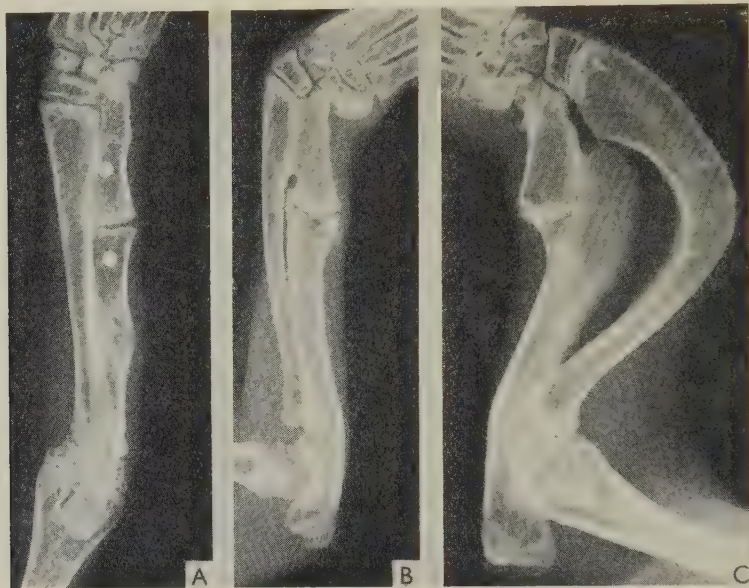
Text-fig. 1. Cross-union between radial shaft and ulnar epiphysis has produced dislocation of the radius at the elbow.

*(b) Reimplantation in reversed position*

The measurement of the radiographs of the nineteen animals falling into this group are recorded in Table 2. Animals followed for more than 2 weeks showed a shortening which in general increased as the age of the animal advanced. The total postoperative growth of the ulna increased to a maximum of 18.5 mm. This growth included contributions from both proximal and distal ends of the bone. Comparison of the size of the distal epiphysis on both operated and control limbs showed that in each case the epiphysis was longer than its fellow (Text-fig. 2A). Direct comparison of the radiographs showed that the epiphysis was also broader. The increase in size of the distal epiphysis occurred mainly in the first 6 weeks after reversal, and subsequent growth was slight or absent. Since shortening was present throughout the whole series, in no case was the activity of the reversed epiphyseal cartilage as great as that of the normal. Cross-union of radial shaft and ulnar epiphysis was seen in seven animals, but growth of the distal epiphysis was not sufficiently vigorous to produce a complete dislocation at the elbow (Text-fig. 2B).



In most animals the epiphysis started to fuse with the shaft after 5 or 6 weeks, although occasionally a thin epiphyseal line was demonstrable until biopsy many weeks later. The retardation of growth was associated with an outward curvature of the radius. Where this curvature of the radius was particularly pronounced, considerable new bone formation was seen on the inner aspect of the curve. It was often associated with a spur of bone on the radial aspect of the ulnar metaphysis (Text-fig. 2C).



Text-fig. 2. A. Elongation of the ulnar epiphysis following reversal of the cartilage. The lead markers were inserted some weeks after operation and do not indicate the rate of bone growth. B. Cross-union between radial shaft and ulnar epiphysis following reversal of ulnar epiphyseal cartilage. Further growth of the ulna has only produced slight displacement of the radius at the elbow. C. Marked shortening of the ulna and curvature of the radius following reversal of the ulnar epiphyseal cartilage.

The contribution of the reversed epiphyseal cartilage to shaft elongation is difficult to assess, although in certain animals the insertion of shaft markers permitted comparisons with the control. During the first two postoperative weeks, the distance between the marker and the epiphyseal cartilage occasionally increased; this effect was always slight, but was a little more conspicuous in the younger animals. No valid shaft growth from the distal epiphyseal cartilage was demonstrable radiologically after this period.

Examination of the sections of these animals demonstrated that union of the bony edges of the reversed fragment with the adjacent bone of the ulna was complete within 2 weeks. Six days after operation the epiphyseal cartilage was united proximally by fibrous tissue to the shaft of the ulna. The cartilage itself was longer than normal, mainly due to an increase in the length of the cartilage columns, but the fragment of metaphysis left attached to the cartilage after the operation was no

longer visible (Pl. 1, fig. 2). Sections from animals killed during the following week showed an increasing irregularity of the cartilage columns, and a patchy re-organization of the epiphyseal bone into a new 'metaphysis'. Irregularity of the epiphyseal cartilage ceased when this 'metaphysis' was fully organized, and by the fifth post-operative week the epiphyseal cartilage had returned to its normal proportions. Sections at this time showed a normal epiphyseal plate, although structurally and functionally reversed. The metaphysis lay on the epiphyseal aspect and gave rise in the usual way to a perichondrial ring (Pl. 1, fig. 3).

Table 2. *Excision of distal ulnar epiphyseal cartilage and reimplantation in reversed position*

No.	Survival (weeks)	Growth (mm.)		Shortening (mm.)	Growth of distal epiphysis (mm.)	
		Operated	Control		Operated	Control
190	1	0.5	1	0.5	*	*
191	1½	2.5	2.5	0	*	*
192	2	1	3	2	*	*
145	3	7	9	2	5	1.5
147	3	6.5	9	2.5	3	1.5
194	5	4.5	7.5	3	4.5	0.5
193	6	6	10.5	4.5	4.5	0
57	8	5.5	13	7.5	4.5	0.5
129	8	12	25	13	7	3
65	9	7.5	15.5	8	7	0.5
78	10	8.5	25	16.5	5.5	1.5
128	10	10	29	19	4.5	3
62	11	10.5	18	7.5	7	0.5
76	11	11.5	26.5	15	8	1
61	13	14.5	31.5	17	9	0.5
67	13	7.5	18	10.5	6.5	0.5
144	20	16	40	24	7.5	1
130	22	14	50.5	36.5	9.5	3
131	22	18.5	46.5	28	10	3

\* Not measured.

In the later sections the epiphyseal cartilage was seen to become narrow, and finally quiescent, although in most animals it still showed a reversal of its normal polarity (Pl. 1, fig. 4). In three animals complete bony fusion appeared to have occurred between epiphysis and diaphysis.

#### DISCUSSION

The growth which occurs after excision and reimplantation of the epiphyseal cartilage was first described by Helferich in 1899. In these experiments the distal epiphyseal cartilage of the ulna of the dog was excised and replaced, and normal growth was observed to follow. The histological changes in Helferich's material were described in some detail by Enderlen (1899). The bone cells attached to the reimplanted fragment died, but the cartilage, although for some time showing a broadening and an irregularity of its structure, remained to contribute to the normal growth in this area. No growth was detected if the epiphyseal cartilage was boiled in physiological saline before reimplantation. The studies of Helferich were amplified by Heller (1914, 1917) who also described the enlargement of the epiphysis which follows reversal of the epiphyseal cartilage. A further contribution to this problem was made by Sousa

Pereira (1937) who confirmed the earlier observations and also demonstrated the failure of the epiphyseal cartilage to grow when transplanted to the shaft of the ulna. The experiments of Haas, however, which were first reported in 1915, and later repeated in 1931, show no growth on reimplantation or reversal of the cartilage. Haas suggests that the elongation after operation may be due to incomplete removal of the epiphyseal cartilage, but the weight of the evidence is clearly against this view.

In an examination of the growth which follows simple reimplantation of the epiphyseal cartilage it is apparent that the operation causes little disturbance of bone growth. Since growth does not follow if the epiphyseal cartilage is boiled prior to reimplantation, it would appear that it is the original cartilage which contributes to bone elongation. This view is confirmed by the histological studies which show persistence of the original transplant, and no evidence of cartilage regeneration.

If the epiphyseal cartilage is replaced in the reversed position, it retains for some time its original polarity, and an enlarged bony epiphysis is produced. The well-organized 'metaphysis' which is produced within the epiphysis suggests that this region is organized by the epiphyseal cartilage, or at least that its structure is determined by the activities of the cartilage. Most of the animals used in these experiments were of an age in which the epiphyseal cartilage has lost its original bipolarity, but in three animals aged 3 weeks at operation there was no evidence that the reversed epiphyseal cartilage made any significant contribution to shaft elongation. Whilst reversal of the epiphyseal cartilage allows growth in length to continue, the rate of growth is always slower than that of the control, and terminates after 6-8 weeks. There is no direct evidence in these experiments to suggest a constant cause for the cessation of growth. Cross-union between radial shaft and ulnar epiphysis is common, and leads to considerable compression of the ulnar epiphyseal cartilage. It has been shown by Blount & Clarke (1949) that pressure across the epiphyseal cartilage restricts its growth, but animals in which cross-union did not occur still failed to grow after the first few weeks. It may well be that the blood supply of the distal epiphysis of the ulna is inadequate to maintain the nutrition of the cartilage and of the new 'metaphyseal' area. As the epiphysis increases in size its cartilage retreats further from the vessels which supply it. Examination of the radiographs suggests that the epiphysis attains a length of 12-16 mm. under these conditions before growth stops.

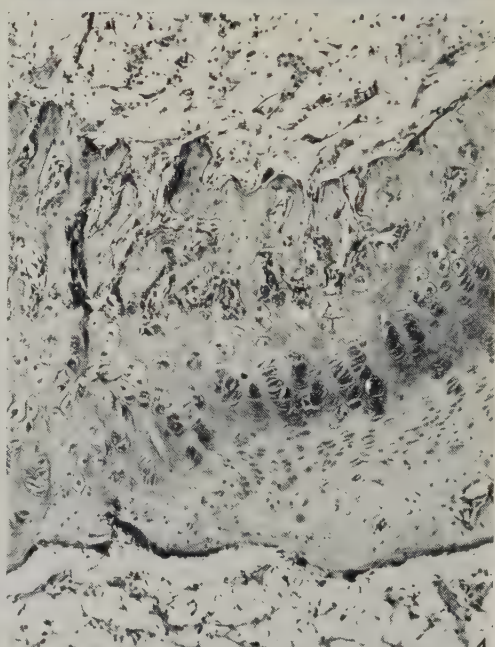
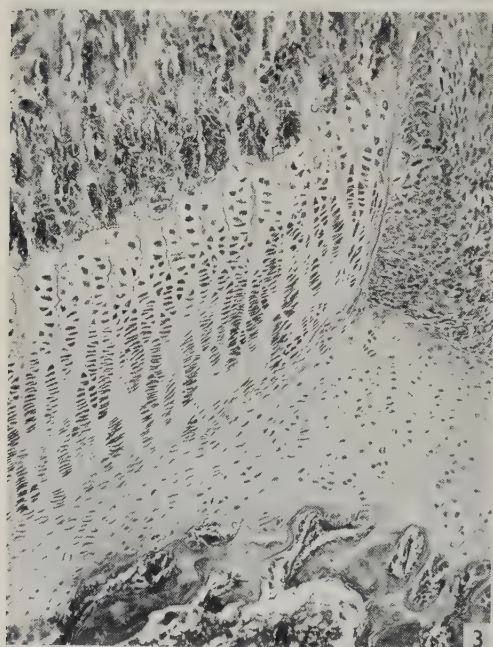
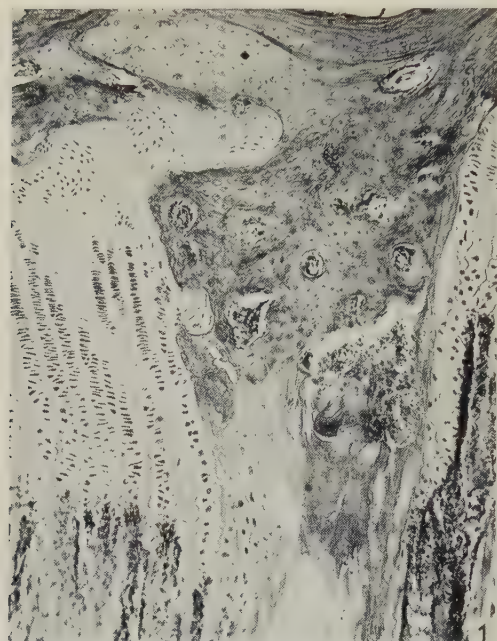
These experiments confirm that the activities of the epiphyseal cartilage are largely predetermined, and that its polarity is determined by its structure rather than its site. Whilst the mode of growth is uninfluenced by changes in its orientation, the rate of growth is restricted when the cartilage is reversed.

#### SUMMARY

1. The effect upon bone growth of excision and reimplantation of the epiphyseal cartilage has been studied.
2. If the epiphyseal cartilage is replaced in its normal position, the subsequent growth of the bone is undisturbed.
3. Excision and reversal of the epiphyseal cartilage leads to enlargement of the bony epiphysis, the cartilage retaining its original polarity.







RING—EXCISION AND REIMPLANTATION OF EPIPHYSEAL CARTILAGE OF THE RABBIT

(Facing p. 237)

4. Growth of the bone following reversal of the epiphyseal cartilage is slow, and ceases after 6-8 weeks.

I wish to acknowledge with gratitude the encouragement and constructive criticism of Prof. W. J. Hamilton. I am indebted to Mr R. J. McCulloch for his technical assistance and to Mr E. V. F. Pittcock for the photography.

#### REFERENCES

- BLOUNT, W. P. & CLARKE, G. R. (1949). Control of bone growth by epiphyseal stapling. *J. Bone Jt. Surg.* **31A**, 464-478.
- BOEREMA, I. (1942). Über das Knochenwachstum. *Acta neerl. morph.* **4**, 365-377.
- ENDERLEN, E. (1899). Zur Reimplantation des resezierten Intermediärknorpels beim Kaninchen. *Dtsch. Z. Chir.* **51**, 574-598.
- GOTTESLEBEN, in SCHINZ, H. R., BAENSCH, W. & FRIEDL, E. (1939). *Lehrbuch der Röntgendiagnostik*, **1**, 63-65. Leipzig: G. Thieme.
- HAAS, S. L. (1915). The experimental transplantation of the epiphysis with observation on the longitudinal growth of bone. *J. Amer. Med. Ass.* **65**, 1965-1971.
- HAAS, S. L. (1931). Further observation on transplantation of the epiphyseal cartilage plate. *Surg. Gynec. Obstet.* **52**, 958-963.
- HELFERICH, H. (1899). Versuche über die Transplantation des Intermediärknorpels wachsender Röhrenknochen. *Dtsch. Z. Chir.* **51**, 564-573.
- HELLER, E. (1914). Experimentelle Untersuchungen über die Transplantation des Intermediärknorpels in Form der halbseitigen Gelenktransplantation. *Arch. klin. Chir.* **104**, 843-954.
- HELLER, E. (1917). Versuche über die Transplantation Knorpelfuge. *Arch. klin. Chir.* **109**, 1-62.
- LACROIX, P. (1951). *The Organization of Bones*. London: Churchill.
- PAYTON, C. G. (1933). The growth of the epiphyses of the long bones in the madder-fed pig. *J. Anat., Lond.*, **67**, 371-381.
- SIEGLING, J. A. (1941). Growth of the epiphyses. *J. Bone Jt. Surg.* **23**, 23-36.
- SOUSA PEREIRA (1937). Le renversement du cartilage du conjugaison et son influence sur le travail d'ossification enchondrale et la croissance de l'os. *Lyon chir.* **34**, 513-541.

#### EXPLANATION OF PLATE

All sections are displayed with the distal end of the ulna to the top of the plate.

- Fig. 1. The reimplanted epiphyseal cartilage 11 weeks after operation. The centre of the cartilage is ossified, but this bone does not completely unite epiphysis and metaphysis.  $\times 80$ .
- Fig. 2. Six days after reversal of the epiphyseal cartilage. The cartilage columns are long and irregular, and are calcified distally.  $\times 72$ .
- Fig. 3. The periphery of the epiphyseal cartilage 5 weeks after reversal. Reversal of structure and function now appears complete. The metaphysis lies on the perichondrial epiphyseal aspect of the cartilage. The perichondrial ring is continuous with the periosteum of the epiphysis.  $\times 96$ .
- Fig. 4. The epiphyseal cartilage 8 weeks after reversal. The cell columns are still directed towards the epiphysis. Absence of a new 'metaphysis' suggests that growth has ceased and this is confirmed by radiological measurements.  $\times 88$ .



## ALKALINE PHOSPHATASE ACTIVITY IN THE DEVELOPING TEETH OF THE RAT

By N. B. B. SYMONS

*Dental School, University of St Andrews, Dundee*

Since Gomori (1939) introduced his well-known method for the demonstration of alkaline phosphatase in histological sections, a number of investigations have been carried out, using this method or some modification of it, on the distribution of alkaline phosphatase in developing teeth. Though these investigations agree on the general picture of alkaline phosphatase activity in developing teeth there has been considerable discrepancy about the detailed distribution of the enzyme, particularly regarding its appearance in the odontoblasts and ameloblasts.

Within recent years the use of the simultaneous coupling azo-dye method has been employed as an alternative method for the demonstration of alkaline phosphatase. The original method was designed by Menten, Junge & Green (1944); but since then a number of more easily performed and superior modifications have been described. The simultaneous coupling azo-dye method serves not only as a check on Gomori's method but appears to have certain distinct advantages. The azo-dye produced, if a suitable coupling agent is employed, is of a particulate nature and so the diffuse staining which was always a disadvantage of the Gomori method is avoided. Moreover, there is no nuclear staining with the simultaneous coupling method, this is a feature of the Gomori method and has in a great number of instances been definitely proved to be a diffusion artifact (Martin & Jacoby 1949; Novikoff, 1951). Finally, the reaction can be watched as it develops and so can be stopped at whatever point desired. It was therefore felt that the use of this method might produce a more accurate picture of alkaline phosphatase distribution in developing teeth than the methods employed previously.

Both rat incisors and first molars have been studied from the time at which the tooth-germs first show a clearly differentiated internal enamel epithelium as a layer of columnar cells.

### MATERIAL AND METHODS

Rat foetuses from 18 days to full term were obtained from animals whose pregnancies were dated from the finding of spermatozoa in a vaginal smear. Post-natal rat material was also employed. It was found that quite good sections could be obtained up to 4 days after birth without decalcification, but after that time it was impossible to obtain intact sections. Material which was to be embedded in paraffin wax was fixed in chilled absolute alcohol, while for frozen sections the material was fixed in chilled formalin. The simultaneous coupling azo-dye technique used is that given by Pearse (1953). Both sodium  $\alpha$ - and  $\beta$ -naphthyl phosphate were tried as substrate but the sodium  $\alpha$ -naphthyl phosphate gave by far the best results since the azo-dye produced is much more insoluble in water than that with sodium  $\beta$ -naphthyl phos-

phate. All observations recorded are based on the use of the sodium  $\alpha$ -naphthyl phosphate. The coupling agents employed were the diazonium salts of 4 chloro-*o*-anisidine and 5 chloro-*o*-toluidine. A longer reaction time is required when the latter is employed, but there is a less degree of background staining. Otherwise they gave similar results. Some sections from each stage were also treated by Gomori's calcium-cobalt method for comparison.

#### OBSERVATIONS

##### *Eighteen-day foetal rat*

The tooth-germs of the incisors which have reached the cap stage of the enamel organ show a distinct layer of columnar cells representing the internal enamel epithelium. There is no sign of odontoblast formation in the dental papilla. There is some alkaline phosphatase activity in the stellate reticulum, particularly in the part adjacent to the internal enamel epithelium, that is in those cells which will give rise to a distinct stratum intermedium. But it is small in amount compared with that of the surrounding alveolar bone. An extremely faint staining is shown by the dental papilla (Pl. 1, fig. 1).

##### *Nineteen-day foetal rat*

The enamel organ of the incisors is now approaching the bell stage, particularly in the lower jaw; and in the lower incisor the stellate reticulum has almost disappeared except at the growing end of the tooth-germ and over the incisal tip of the tooth. There is some indication of the appearance of odontoblasts in the dental papilla. There is now a greater degree of alkaline phosphatase activity in the enamel organ, particularly in the stratum intermedium, but not at all in the cells of the internal enamel epithelium. It is very largely confined to the enamel organ at the incisal tip and on the labial aspect of the tooth-germ. It is of almost equal intensity to that of the surrounding bone. A greater degree of staining is now shown by the dental papilla particularly near the incisal part of the tooth-germ (Pl. 1, fig. 2).

##### *Twenty-day foetal rat*

Everywhere, except at the growing base of the incisors, the stellate reticulum has disappeared so that over almost the whole of the tooth the enamel organ is present in a reduced form. There is heavy staining of the stratum intermedium of an intensity now at least equal to that of the surrounding alveolar bone. The other cells of the enamel organ external to the stratum intermedium show a lesser degree of alkaline phosphatase activity. This staining of the reduced enamel organ is confined to the labial aspect of the developing tooth. The internal enamel epithelium is everywhere unstained, except for a number of linear streaks passing from stratum intermedium to odontoblast layer. A distinct layer of fully differentiated odontoblasts is present over a great area of the pulp (dental papilla), particularly on the labial aspect of the tooth, and in these parts dentine formation has begun, a thin layer of uncalcified matrix being present. The pulp tissue, and especially that part deep to the layer of odontoblasts, shows marked alkaline phosphatase activity though of not quite the same intensity as that of the stratum intermedium. The odontoblasts stain also, but to a less degree than the pulp tissue. The alkaline phosphatase activity is not confined

to the cytoplasm of the pulp cells and the odontoblasts as dye particles can be seen heavily arranged along the course of the pulp fibres and especially along the fibres of von Korff (Pl. 1, fig. 3).

The first molars are now at the stage which was reached by the incisors on the eighteenth day and show a similar distribution of alkaline phosphatase activity.

#### *Full term rat*

At this time dentine formation is progressing over a wide area of both labial and lingual aspects of the incisors, though the dentine is rather thicker labially than lingually; calcification of the dentine has begun, distinct dentine and pre-dentine layers being distinguishable. Formation of enamel is also present, being confined, of course, to the labial aspect of the tooth, and in this area the cells of the internal enamel epithelium have become fully differentiated ameloblasts (Pl. 1, fig. 4). Alkaline phosphatase activity is most intense in the stratum intermedium, the rest of the reduced enamel organ shows a somewhat less degree of staining. The ameloblasts show no enzyme activity except in the narrow basal part of the cytoplasm, i.e. next to the stratum intermedium. This is not always easily appreciated in paraffin sections but is very obvious with the greater accuracy of enzyme distribution given by frozen sections (Pl. 1, fig. 5). On the pulpal aspect the greatest alkaline phosphatase activity is shown by a band of pulp tissue immediately deep to the odontoblasts; this band of pulp tissue is broader on the labial than on the lingual aspect of the incisor. This zone of the pulp is characterized by the numerous cells present in it, so that even in a routinely stained section it is distinct from the rest of the pulp and bears a relationship to the odontoblasts almost like that of the reduced enamel organ to the ameloblasts. The odontoblasts show a lesser degree of staining. As in the previous stage the dye particles are not only in the cytoplasm of the odontoblasts and the general pulp cells but are also extracellular being particularly heavily distributed along the course of the pulp fibres and fibres of von Korff. The general pulp tissue shows only a very slight amount of staining (Pl. 1, fig. 5).

In the first molar formation of dentine has begun. The distribution of alkaline phosphatase activity is the same as in the incisors at a comparable stage, i.e. at approximately 20 days foetal life; except that the enamel organ of the molars is in an unreduced form and there is some staining of the stellate reticulum.

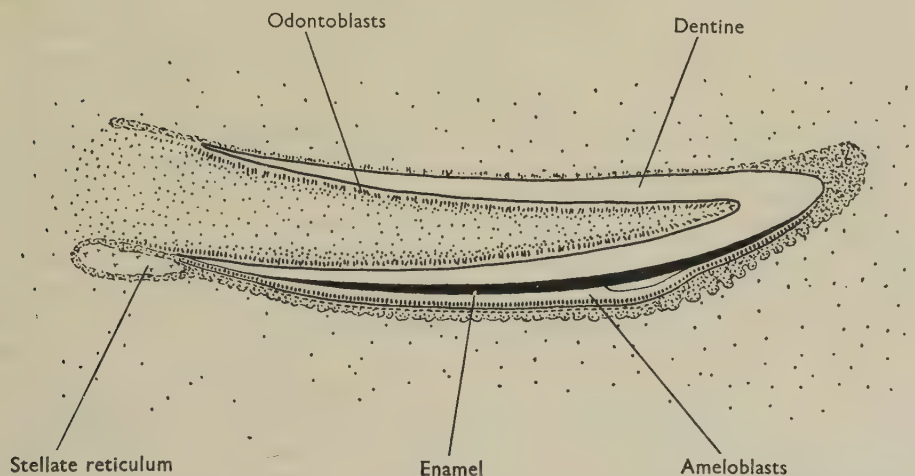
#### *Four-day rat*

Over the greater part of the incisors the pattern of alkaline phosphatase distribution is exactly the same as that of the preceding stage. However, there is one very notable difference in that towards the incisal tip of the tooth the ameloblasts, which were previously unstained except in the narrow basal area, now show an increasing amount of alkaline phosphatase activity throughout the whole of their cytoplasm. The point at which this generalized staining of the ameloblasts first appears coincides exactly with the region at which the previously highly columnar ameloblasts commence to shorten considerably. Just apical to this region there is an almost constant artifact in the form of a tearing away of the ameloblasts from the surface of the enamel (Text-fig. 1). This has been pointed out previously by Marsland (1951).



The staining of the ameloblasts increases as one proceeds incisally so that just short of the incisal tip where the enamel ceases the ameloblasts show a degree of alkaline phosphatase activity which is about equal to that of the reduced enamel organ (Pl. 1, fig. 6).

The first molars have now reached approximately the same stage of development as was shown by the incisors at full term. That is formation of both dentine and enamel is progressing but no shortening of the ameloblasts has yet occurred. The



Text-fig. 1. A diagrammatic representation of a lower incisor of a 4-day-old rat. The alteration in the length of the ameloblasts which occurs towards the tip of the tooth, and the artifact which commonly occurs at this region should be noted. External to the ameloblasts the enamel organ shows no stellate reticulum; it is in a reduced form.

pattern of alkaline phosphatase distribution is exactly the same as that of the incisor at full term, except that in the molar, where the enamel organ is unreduced staining, apart from the intense reaction of the stratum intermedium is, in a weaker form, also found across almost the whole width of the stellate reticulum fading out just short of the external enamel epithelium (Pl. 1, fig. 7).

#### *Six-day rat*

Though the sections of incisor of the same standard as that of the 4-day-old material could not be obtained yet it was possible to realize that the pattern of alkaline phosphatase distribution was the same as that of the incisors from 4-day-old rats. As before, where the ameloblasts became reduced in length they showed staining throughout the whole of the cytoplasm.

In the molars the same pattern was now present. Towards the occlusal surface of the cusps the ameloblasts were becoming reduced and showed alkaline phosphatase activity throughout the whole of the cytoplasm.

## DISCUSSION

There is already fairly general agreement amongst previous workers that, after formation of dentine and enamel has started, considerable alkaline phosphatase activity is to be found in the stratum intermedium, in the zone of pulp tissue immediately beneath the odontoblasts, in the external enamel epithelium of the incisors, and to a lesser extent in the stellate reticulum of the molars. This in effect is the view expressed by Engel & Furuta (1942), Gomori (1943), Bevelander & Johnson (1945), Morse & Greep (1947), and Greep, Fischer & Morse (1948); that is, by those investigators who have studied the total pattern of alkaline phosphatase activity in the developing tooth after the appearance of both dentine and enamel. The present study supports this picture of alkaline phosphatase distribution.

Of the above investigators only Engel & Furuta (1942) and Gomori (1943) claim that the odontoblasts show no phosphatase activity, though Gomori states that the odontoblasts occasionally showed staining. On the other hand, Horowitz (1942), Bevelander & Johnson (1946), and Johnson & Bevelander (1954) report the odontoblasts as being phosphatase positive. The present investigation supports the view that the odontoblasts invariably show alkaline phosphatase activity.

As regards the ameloblasts Bevelander & Johnson (1945), Greep *et al.* (1948), Bevelander & Johnson (1949), and Johnson & Bevelander (1954) state that these cells show alkaline phosphatase activity which, according to Morse & Greep (1947), is confined to the nucleus and 'supra-nuclear' (basal) area. Gomori (1943) maintains that the ameloblasts do not usually stain, and Engel & Furuta (1942) claim that the ameloblasts are phosphatase negative. It has been shown in this investigation that while the ameloblasts remain in a highly columnar form they are phosphatase negative except for the small basal area of the cytoplasm next to the stratum intermedium, but that from the stage at which the ameloblasts become reduced in length they begin to show alkaline phosphatase activity.

It has been shown recently by Marsland (1951, 1952) that in the teeth of the rat, the phase during which the ameloblasts remain highly columnar is associated with the production of the enamel matrix and its initial light calcification, but that at the region where the ameloblasts commence to shorten the enamel is beginning to enter the phase of maturation. During this phase the enamel gradually alters from its earlier lightly calcified form to the extremely highly calcified adult enamel. This maturation phase is characterized not only by the shortening of the ameloblasts but also by certain other morphological changes in the enamel organ, notably the disappearance of an easily distinguishable single layer of flattened cells representing the stratum intermedium. The cells adjacent to the basal ends of the ameloblasts become indistinguishable from the cells of the external enamel epithelium and are connected to them by cytoplasmic bridges. Moreover, in the maturation zone the enamel organ develops prominent papillae. All these changes are to be found in the region where the whole cytoplasm of the ameloblasts shows alkaline phosphatase activity. The enamel organ papillae are not very prominent at the 4-day stage in the incisor but there is no doubt of their presence at the 6-day stage.

Apart from Bevelander & Johnson (1945), Bevelander & Johnson (1949), and Johnson & Bevelander (1954), who used mainly pig embryos, all the other investi-

gators describing alkaline phosphatase activity in relation to the ameloblasts, used rat material. Of this latter group only Greep *et al.* (1948) claim that the whole of the ameloblast shows staining. This work was carried out on 28-day rats; at this age the ameloblasts are over a considerable area in the shortened form in which they will give a positive reaction. As regards the highly columnar form of ameloblast associated with enamel matrix formation, the reaction of the ameloblast layer shown in their fig. 4 of the basal formative end of a 28-day-old upper incisor seems to be of doubtful interpretation. The other workers of this group all used rat material of not greater age than 4 days. Since even at 4 days the shortened ameloblasts are only found in the incisors and only over a small area towards the tip of the tooth it is perhaps not surprising that the ameloblasts were reported as not staining. Morse & Greep (1947) state that the nucleus and 'supranuclear' (basal) part of the ameloblasts showed enzyme activity, this agrees with the present study regarding the unshortened ameloblasts; since the nuclear staining of the Gomori method employed was presumably an artifact. Engel & Furuta (1942) and Gomori (1943) claim that the ameloblasts show no staining; but it has been pointed out earlier that the staining of the basal area of the unshortened ameloblasts is not readily seen in paraffin sections, yet is easily observable in frozen ones. In both these investigations paraffin-embedded material was used. It may be stated here that the same difference has been noticed between paraffin and frozen sections of similar material treated with Gomori's calcium-cobalt method.

In the present state of our knowledge regarding alkaline phosphatase it is almost impossible to decide what the exact significance of the general pattern of alkaline phosphatase distribution may be. Though it is generally accepted that alkaline phosphatase is involved in calcification, yet it is now widely held that alkaline phosphatase may also be concerned in the production of ribonucleic acid, in the manufacture of fibrous proteins and in the differentiation of cells; all these processes occur in the developing tooth and mostly at the same time. However, it is worth noting that alkaline phosphatase activity is present in the developing tooth at a stage in foetal life when neither calcification nor the formation of the organic matrices of dentine or enamel has commenced. This would suggest that this enzyme activity is related to the active cell differentiation which occurs in the early developing tooth. This is supported by the continued presence of alkaline phosphatase activity at the growing base of the incisor where no calcification or organic matrix formation is found. This enzyme activity has a pattern like that of the incisor at the 19-day foetal stage and as shown in Pl. 1, fig. 2.

It is very possible that the appearance of alkaline phosphatase activity in the shortened ameloblasts associated with the maturation of the enamel may be related to the final heavy calcification of the enamel which takes place during this phase.

#### SUMMARY

1. Alkaline phosphatase activity has been studied in the developing incisors and first molars of the rat from 18 days foetal life to 6 days after birth, using principally the simultaneous coupling azo-dye method.

2. In the early stages before odontoblasts or ameloblasts have appeared, alkaline phosphatase activity is found in the stellate reticulum and stratum intermedium in



those regions where the enamel organ will produce enamel, and to a less extent in the dental papilla.

3. After formation of dentine and enamel has commenced, alkaline phosphatase activity is found most intensely in the stratum intermedium; to a somewhat less degree in the external enamel epithelium of the incisors, and in a zone of pulp tissue immediately deep to the odontoblasts; and to a still less degree in the stellate reticulum of the molars and in the odontoblasts. The general pulp tissue shows only very slight staining. At this stage and until maturation of the enamel begins the ameloblasts show no staining except at their basal ends, i.e. next to the stratum intermedium.

4. When the phase of enamel maturation is reached, the ameloblasts begin to show alkaline phosphatase activity throughout the whole of the cytoplasm. It has been suggested that this activity may be associated with the final heavy calcification of the enamel which takes place then.

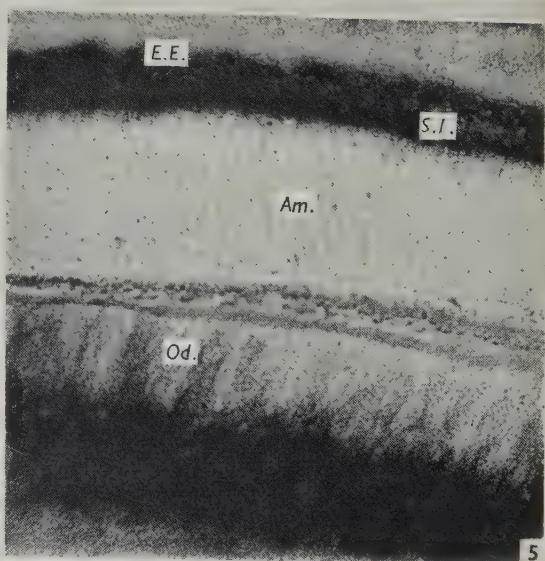
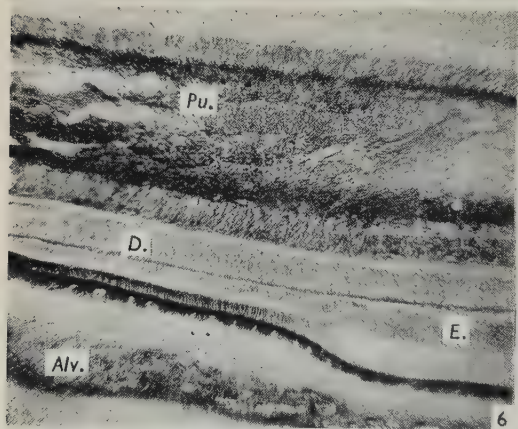
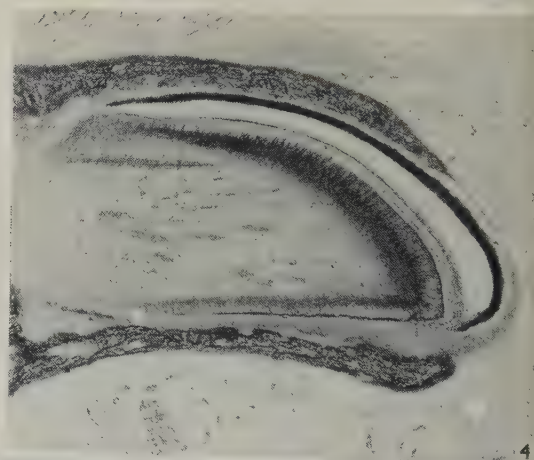
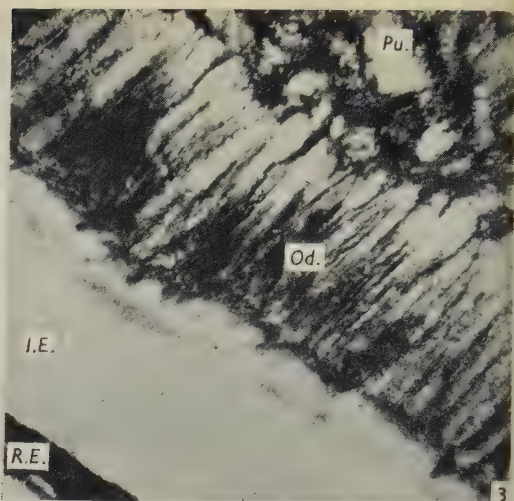
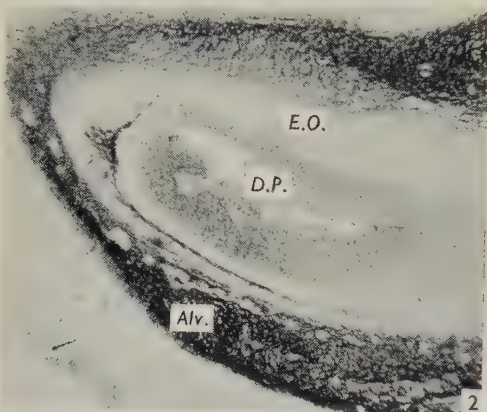
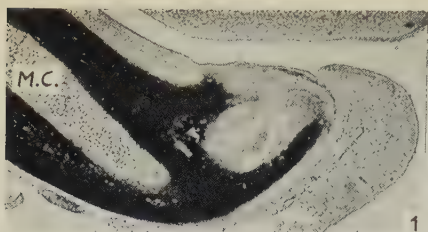
5. It has been suggested that the alkaline phosphatase activity found in the very early stages of tooth development may be concerned in cell differentiation.

I wish to express my thanks to Prof. J. J. Pritchard for much valuable advice. I am also greatly indebted to Imperial Chemicals Industries Ltd. for the diazonium salts employed in this study.

#### REFERENCES

- BEVELANDER, G. & JOHNSON, P. L. (1945). The histochemical localization of alkaline phosphatase in the developing tooth. *J. cell. comp. Physiol.* **26**, 25-33.
- BEVELANDER, G. & JOHNSON, P. L. (1946). Odontoblasts and dentinogenesis. *J. dent. Res.* **25**, 381-385.
- BEVELANDER, G. & JOHNSON, P. L. (1949). Alkaline phosphatase in amelogenesis. *Anat. Rec.* **104**, 125-135.
- ENGEL, M. B. & FURUTA, W. (1942). Histochemical studies of phosphatase distribution in the developing tooth of the albino rat. *Proc. Soc. exp. Biol., N.Y.*, **50**, 5-9.
- GOMORI, G. (1939). Microtechnical demonstration of phosphatase in tissue sections. *Proc. Soc. exp. Biol., N.Y.*, **42**, 23-26.
- GOMORI, G. (1943). Calcification and phosphatase. *Amer. J. Path.* **19**, 197-209.
- GREEP, R. O., FISCHER, C. J. & MORSE, A. (1948). Alkaline phosphatase in odontogenesis and osteogenesis and its histochemical demonstration after demineralization. *J. Amer. dent. Ass.* **36**, 427-442.
- HOROWITZ, N. H. (1942). Histochemical study of phosphatase and glycogen in foetal heads. *J. dent. Res.* **21**, 519-527.
- JOHNSON, P. L. & BEVELANDER, G. (1954). The localization and interrelation of nucleic acids and alkaline phosphatase in the developing tooth. *J. dent. Res.* **33**, 128-135.
- MARSLAND, E. A. (1951). A histological investigation of amelogenesis in rats. I. Matrix formation. *Brit. dent. J.* **91**, 251-261.
- MARSLAND, E. A. (1952). A histological investigation of amelogenesis in rats. II. Maturation. *Brit. dent. J.* **92**, 109-119.
- MARTIN, B. F. & JACOBY, F. (1949). Diffusion phenomenon complicating the histochemical reaction for alkaline phosphatase. *J. Anat., Lond.*, **83**, 351-363.
- MENTEN, M. L., JUNGE, J. & GREEN, M. H. (1944). A coupling histochemical azo dye test for alkaline phosphatase in the kidney. *J. biol. Chem.* **153**, 471-477.
- MORSE, A. & GREEP, R. O. (1947). Alkaline glycerophosphatase in the developing teeth of the rat. *Anat. Rec.* **99**, 379-395.
- NOVIKOFF, A. B. (1951). The validity of histochemical phosphatase methods on the intracellular level. *Science*, **113**, 320-325.
- PEARSE, A. G. E. (1953). *Histochemistry*. London: Churchill.







## EXPLANATION OF PLATE

<i>Alv.</i> Alveolar bone	<i>I.E.</i> Internal enamel epithelium
<i>Am.</i> Ameloblasts	<i>M.C.</i> Meckel's cartilage
<i>D.</i> Dentine	<i>Od.</i> Odontoblasts
<i>D.P.</i> Dental papilla	<i>Pu.</i> Pulp
<i>E.</i> Enamel	<i>R.E.</i> Reduced enamel organ
<i>E.E.</i> External enamel epithelium	<i>S.I.</i> Stratum intermedium
<i>E.O.</i> Enamel organ	<i>S.R.</i> Stellate reticulum

The staining in the sections is due solely to the azo-dye produced as a result of the simultaneous coupling method employed for the demonstration of alkaline phosphatase activity.

- Fig. 1. Eighteen-day foetal rat. Saggital section of lower jaw. The incisor tooth germ is surrounded by the heavy staining of the alveolar bone, and Meckel's cartilage is similarly outlined by bony staining. Paraffin section. Diazonium salt of 5-chloro-*o*-toluidine; reaction time 12 min.  $\times 28$ .
- Fig. 2. Nineteen-day foetal rat. Saggital section of lower incisor and surrounding alveolar bone. Paraffin section. Diazonium salt of 5-chloro-*o*-toluidine; reaction time 12 min.  $\times 70$ .
- Fig. 3. Twenty-day foetal rat. Saggital section of lower incisor, showing region where a thin layer of dentine matrix (predentine) has been formed. The absence of nuclear staining of the odontoblasts should be noted. Paraffin section. Diazonium salt of 5-chloro-*o*-toluidine; reaction time 14 min.  $\times 580$ .
- Fig. 4. Full-term rat. Saggital section of upper incisor and surrounding alveolar bone. Frozen section. Diazonium salt of 4-chloro-*o*-anisidine; reaction time 2 min.  $\times 30$ .
- Fig. 5. Full-term rat. High-power view of a part of the developing incisor seen in fig. 4. The region shown is approximately half-way along the upper (outer) border of the tooth. Between the ameloblasts and odontoblasts thin layers of enamel, dentine, and predentine may be seen.  $\times 260$ .
- Fig. 6. Four-day rat. Saggital section of lower incisor, showing the part towards the tip of the tooth where the ameloblasts change from the highly columnar to the shortened form. The tearing away of the ameloblasts from the surface of the enamel near this region is an almost constant artifact. Paraffin section. Diazonium salt of 5-chloro-*o*-toluidine; reaction time 14 min.  $\times 70$ .
- Fig. 7. Four-day rat. Saggital section of upper first and second molars. Enamel and dentine formation has commenced in the first molar. Frozen section. Diazonium salt of 5-chloro-*o*-toluidine; reaction time 5 min.  $\times 18$ .

## THE ARRANGEMENT OF THE ANSA SPIRALIS OF THE SHEEP COLON

BY R. N. SMITH

*Department of Veterinary Anatomy, The University, Bristol*

Although the general arrangement of the viscera of the sheep is quite well known and described, many details seem to be lacking in the literature. This work is an attempt to supply such information on one region of the large intestine.

The colon of the domestic mammals may be divided for descriptive purposes into three major parts: *colon primum*, *colon secundum* and *colon tertium*. These are considered respectively the homologues of the ascending, transverse and descending parts of the colon of man (Zietzschmann, 1925). The colon primum of the sheep is itself separable into three regions; ansa proximalis, ansa spiralis and ansa distalis. (For this and other anatomical features of the colon primum, described below, see Fig 1.)

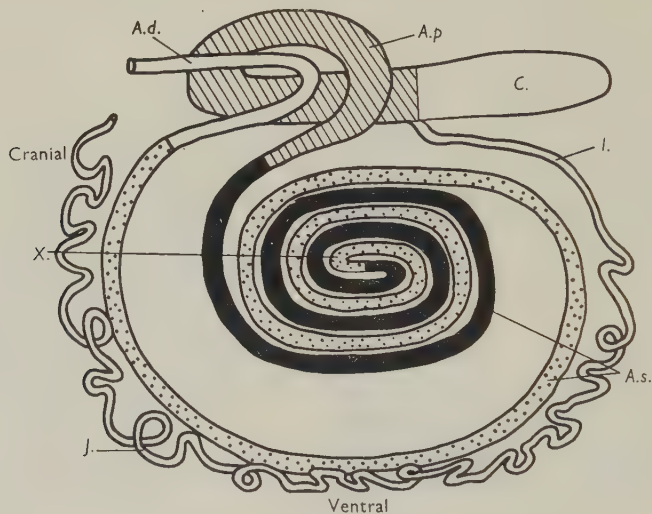


Fig. 1. Diagram of the arrangement of part of the alimentary tract of the sheep. *A.d.*, ansa distalis; *A.p.*, ansa proximalis; *A.s.*, ansa spiralis (centripetal coils solid, centrifugal coils dotted); *C.*, caecum; *I.*, ileum; *J.*, jejunum; *X.*, flexura centralis.

The ansa proximalis is directed cranially from its junction with the caecum; the latter is usually on the right side of the body ventral to the last few lumbar vertebrae with its blind end directed towards the pelvis. The initial part of the ansa proximalis becomes sharply reflected on to itself and then runs caudally for a short distance before another flexion again alters its course in a cranial direction. It is then continued into the ansa spiralis.

This part of the colon primum begins by coiling centripetally. At the centre of the coil the colon sharply reverses direction and runs centrifugally between the centripetal windings. All the coils lie very close to one another except the final centrifugal one which is separated from the main mass by a space containing lymph nodes. The final centrifugal coil ends cranial to the start of the first centripetal coil.

The alimentary tract now continues as the ansa distalis of the colon primum and is topographically closely associated with part of the ansa proximalis, running first caudally and then cranially. When lateral and slightly cranial to the root of the *arteria mesenterica cranialis* it is continued by the colon secundum. The ansa spiralis is embedded in the common mesentery of the jejunum and ileum which are lying in an arc on the circumference.

The above description agrees with those of writers who deal with the topography of the sheep intestine more fully (e.g. Kolda, 1931; Martin & Schauder, 1938; Ackerknecht, 1943). However, there is little agreement between the various accounts of the number of coils forming the ansa spiralis. Martin & Schauder (1938) and Ackerknecht (1943) say there are 'mostly' three centripetal and an equivalent number of centrifugal coils. Applying the method used in this article (see below) this appears to correspond to three centripetal and three and a half centrifugal coils. Fig. 782 of Ackerknecht (1943) does not accord with the description in his text as it shows two and a half centripetal and three centrifugal coils (our method). Kolda (1931) describes the alimentary tracts of three sheep with three centripetal and three and a half centrifugal coils, and of four sheep with three and a half centripetal and four centrifugal coils (our method). Hafner (1909), in his dissertation describing the development of the sheep intestine, mentions a 10.5 cm. crown-rump foetus and a 15 cm. crown-rump foetus with three and a half centripetal and an equal number of centrifugal coils. Martin & Schauder (1938) and Ackerknecht (1943) give the additional information that irregularities sometimes occur in the coiling.

Preliminary observations on class material indicated that a variety of patterns exists. It was therefore decided to examine a large number of specimens to record the various patterns of the ansa spiralis and how frequently each occurred.

#### MATERIALS AND METHODS

One thousand and sixty one sets of sheep intestines were examined shortly after removal from the animals. A diagram was made of the arrangement of the left side of the ansa spiralis. There was no attempt to record the size of the various parts, attention being solely directed to the conformation.

The ansa spiralis was considered to start and end at points level with the dorsal limit of the coil. The centripetal coils were counted to the centre of the spiral and the centrifugal coils from this point to the end of the ansa spiralis. This results in a half more centrifugal than centripetal coils. For example, the specimen in Fig. 1 is considered to have three centripetal and three and a half centrifugal coils. Another method of counting relies on imagining the colon as a loop of bowel like a very elongated 'U', coiled on to itself. The bottom of the 'U' (point X, Fig. 1) is called the flexura centralis. The number of coils of this doubled piece of colon is assumed to give the number of centripetal and centrifugal coils. The first method always gives



the same number of centripetal coils but gives a half centrifugal coil more than the second method. We have used the first method in this article.

It was not possible to relate the tracts to specific sheep, and so no record of breed or sex was kept. However, as the inspections were spread over several months it is to be expected that several breeds were included in the survey.

### RESULTS

Of the 1061 specimens examined 219 (20.64 %) showed some irregularity, ranging from an S-shaped insertion of varying size in an otherwise normal coil (for example Fig. 2 A) to a complete departure from the spiral arrangement (for example Fig. 2 B). These irregular patterns are not analysed in this paper.



Fig. 2. A, diagram of an ansa spiralis with an S-shaped insertion in an otherwise normal arrangement; B, diagram of an ansa spiralis with a complete departure from the spiral arrangement.

The remainder, 842 or 79.36 %, showed five arrangements in the number of windings in the spiral. The frequency of these patterns is shown in Table 1. The table shows the number of specimens out of a total of 1061 in each of the groups; this number is also expressed as a percentage of the total.

Table 1. *The specimens with regular spiral patterns divided into five groups according to the number of coils in the ansa spiralis*

No. of centripetal windings	No. of centrifugal windings	No. of specimens	Percentage of total examined
2	2½	2	0.19
2½	3	145	13.67
3	3½	516	48.63
3½	4	173	16.31
4	4½	6	0.57

Overlapping of adjacent coils sometimes occurred and in several cases the flexura centralis overlaid part of a nearby winding.

### DISCUSSION

The survey shows that a considerable percentage (20.64 %) of sheep have some irregularity in the coiling of the ansa spiralis. It appears from remarks by authorities such as Martin & Schauder (1938) and Ackerknecht (1943), (e.g. 'irregularities sometimes occur') that the frequency of these irregular patterns has been seriously underestimated.

It is confirmed that three centripetal and three and a half centrifugal coils is the commonest arrangement, thus agreeing with Martin & Schauder (1938) and Ackerknecht (1943), bearing in mind their different method of recording. It is interesting to note that even so this pattern appeared in less than half those examined.

It is hoped that a further survey will be carried out to establish whether breed or sex influences the frequency of the patterns. It is certain that age is of no consequence in the post-natal animal as the coil is too firmly fixed on the common mesentery to allow the pattern to be altered, although in some cases the centre of the coil can be lifted slightly.

#### [SUMMARY

1. A brief description is given of part of the alimentary tract of the sheep with especial reference to the colon primum (homologous with the ascending colon of man).

2. An analysis of the arrangement of the ansa spiralis of 1061 sheep showed that 842 or 79.36 % had a regular spiral.

3. Irregularities ranged from small S-shaped insertions in otherwise normal coils to complete departures from the spiral arrangement.

3. The commonest pattern of the ansa spiralis was three centripetal and three and a half centrifugal coils and this arrangement was found in 48.63 % of all specimens examined.

My thanks are especially due to Mr A. Coombs of this Department for his help in collecting the data. Mr Howick (meat inspector) and the staff of the Bristol Abattoir, Gordon Road, have, as always, been extremely co-operative. I am also grateful to Prof. C. W. Ottaway, Prof. J. M. Yoffey and Mr A. S. King for their advice.

#### REFERENCES

- ACKERKNECHT, E. (1943). Das Eingeweidesystem. In Ellenberger-Baum, *Handbuch der vergleichenden Anatomie der Haustiere*, 18th ed., pp. 448, 449. Berlin: Springer.
- HAFNER, B. (1909). Die Entwicklung der Lage und Anordnung des Schweine- und Wiederkäuerdarmes. Diss. Giessen.
- KOLDA, J. (1931). Zur Topographie des Darmes beim Schaf und bei der Ziege. *Z. ges. Anat.* 1. *Z. Anat. EntwGesch.* 95, 243-269.
- MARTIN, P. & SCHAUDER, W. (1938). *Lehrbuch der Anatomie der Haustiere*, Bd. 3. Anatomie der Hauswiederkäuer. 3rd ed., pp. 192, 193. Stuttgart: Schickhardt und Ebner.
- ZIETZSCHMANN, O. (1925). Der Darmkanal der Säugetiere, ein vergleichend-anatomisches und entwicklungsgeschichtliches Problem. *Anat. Anz.* 60, 155-172.

# THE PART PLAYED BY THE TONGUE IN MASTICATION AND DEGLUTITION

BY SHAFIK ABD-EL-MALEK

*Department of Anatomy, Abbasia Medical Faculty, Egypt*

## INTRODUCTION

The functions of the human tongue in tasting, swallowing and speaking are well known. Its assistance in mastication is also well recognized; Gibbons (1898), for example, states that besides controlling, with the assistance of the buccinator the position of the food between the teeth, it also turns the food and mixes it with saliva and aids in separating and sorting out unsuitable particles.

As regards the individual movements of the tongue, Harris (1927), from radiographic observations using barium milk, sometimes thickened with bread, described movements including pocket formation between the tip of the tongue and the teeth, followed in succession by hollowing of the dorsum, by raising of the anterior half to make contact with the palate and depression of the posterior half, and a final piston-like stroke as the barium was 'shot downwards'. Johnstone (1942) also described the hollowing of the dorsum, but emphasized the forceable and sudden elevation of the hyoid bone and larynx as the tongue was pressed against the palate and the barium projected through the fauces. Whillis (1946) speaks of an anticipatory protrusion of the tongue so that its tip comes into contact with the lower lip, followed by retraction carrying the food into the mouth. But it is clear that these complex movements are not yet fully understood, particularly those associated with the distribution of the food during mastication.

## METHODS OF INVESTIGATION

Observations were made on subjects who had lost some of their teeth, preferably on both sides, one side then being available for illumination and the other for observation. In such subjects the movements of the tongue could be seen by parting the lips with small retractor forceps, so as to expose the teeth on the side under observation. Nuts, coloured gelatin food stuffs and coloured chewing gums were used, and drawings showing positions of the tongue were made for later elaboration. The range of tongue movements was studied photographically with the mouth open. Radiographic examination proved less successful, chiefly on account of the speed at which mastication was performed. A few observations on hemiplegic patients were included.

## THE SEQUENCE OF MASTICATION AND DEGLUTITION

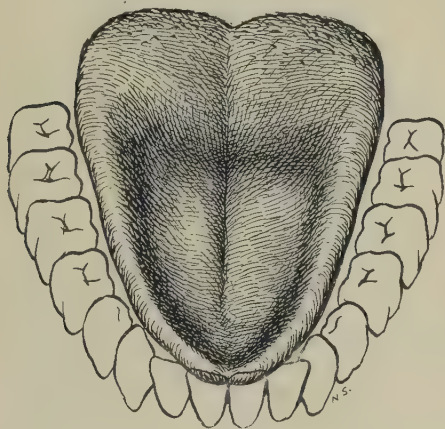
The normal sequence of mastication comprises five stages:

(1) *The 'preparatory' stage.* The tongue being placed in the resting position on the floor of the mouth, with its dorsum looking upwards, prepares itself for the reception of the food by becoming trough-like, so as to collect the foodstuff on its dorsum

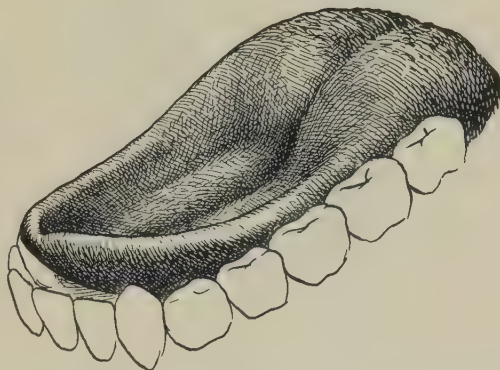


(Text-figs. 1, 2). Pocket formation at the tip and contact with the lip are unusual when solid matter is taken.

(2) *The 'throwing-stage'*. The anterior half, or rather more of the tongue, having collected the foodstuff, twists over on one side, turning through a right angle so that its dorsum is made to face the lingual surface of the teeth (Text-fig. 3). This twisting is carried out by a sudden movement which throws the ingesta on the surface of the lower grinding teeth.



Text-fig. 1.



Text-fig. 2.

Text-fig. 1. A diagram to show the tongue in the mouth surrounded by the teeth of the lower jaw. It shows the tongue in the first or preparatory stage of mastication or trough-like formation.

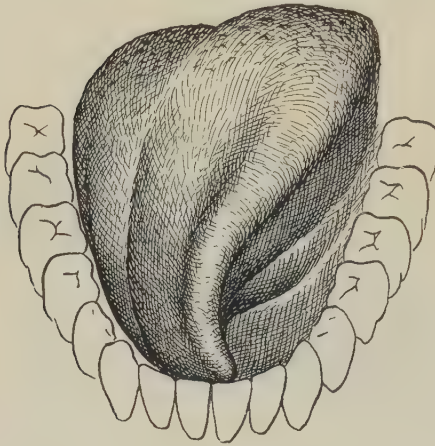
Text-fig. 2. A diagram to illustrate the tongue assuming a well-formed trough to collect the food before the next throwing stage.

(3) *The 'guarding' stage*. The tongue retains the twisted position and its dorsum presses on the medial side of the grinding teeth to prevent the food slipping medially into the buccal cavity (Text-fig. 4). The tongue and the buccinator muscle of the appropriate side act together to keep the food between the grinding teeth, and prevent its escape, while the teeth cut and grind the ingesta.

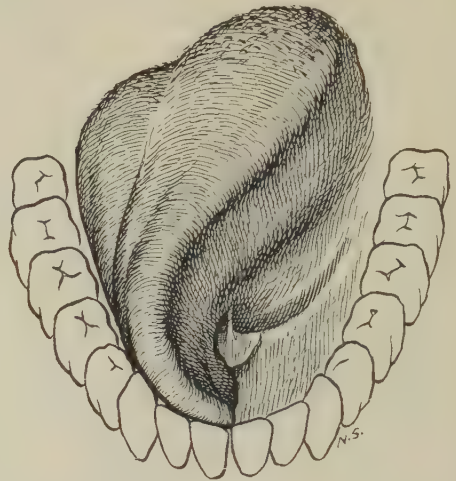
(4) *The 'sorting-out' stage*. This begins when, at the completion of a series of chewing movements, the grinding teeth are separated from one another. The buccinator muscle drives the cheek medially so that it bulges between the teeth and pushes the food into the buccal cavity and on to the tongue, which is now preparing to regain its position of rest on the floor of the mouth. The tongue movement is again rapid and jerky, and this may help to sort out the larger particles of food, which still need crushing, on to the middle trough-like part of the tongue, whence they can be replaced between the teeth, while the parts already sufficiently crushed are placed more laterally on the tongue. These movements are repeated as long as any food particles remain uncrushed.

(5) *The stage of 'bolus formation'*. This partly overlaps the last. The tongue makes alternate side to side churning movements designed to mix the crushed food with saliva, coat it with mucus, and make it into a bolus ready for swallowing. The tongue now prepares itself for the act of swallowing.

(6) In the first stage of deglutition, that of 'closure', the tip of the tongue is raised and pressed rather hard against the posterior surface of the front teeth and the anterior part of the hard palate, so as to close off the mouth and pharynx. In swallowing fluid the tongue assumes at this stage a deep gutter-like form (Fig. 5).



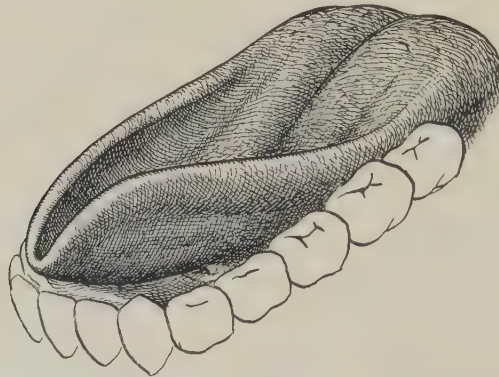
Text-fig. 3.



Text-fig. 4.

Text-fig. 3. A diagram to show the tongue twisting to the right, during the second stage of mastication or the 'throwing stage'.

Text-fig. 4. A diagram of the tongue in the third or 'guarding stage'; the tongue is pressing on the medial side of both the lower and upper teeth. The ventral surface of the tongue with its twisting frenulum is seen.



Text-fig. 5. A diagram to show the tongue in a gutter-like shape during deglutition of fluids.

(7) *Stage of 'slide preparation'.* The hyoid bone is pulled sharply upwards and anteriorly, and the posterior part of the tongue is depressed towards the hyoid bone. The tongue now assumes a special position and form. It slopes obliquely downwards and backwards, its tip is pressed against the front teeth and hard palate, and its root against the hyoid bone. It is rendered concave both from side to side,

especially near the tip, and also antero-posteriorly, so as to present a smooth slope leading from the mouth to the oropharynx.

(8) *Stage of 'pressure'*. The bolus is forced by pressure between the tongue and palate to move backwards towards the isthmus and over the posterior part of the tongue as it enters the pharynx.

#### THE MUSCLES CONCERNED

Depression of the tongue near the mid-line and raising of its lateral margins are produced by contraction of the genio- and hyoglossi combined with bilateral action of the styloglossi (Pl. 1, fig. 1, in which the tongue is also protruded to bring it within view of the camera). When the tip is turned sharply upwards the superior longitudinal muscles come to stand out as a pair of thick parallel bands lying mid-way between the median plane and the lateral margins of the tongue (Pl. 1, fig. 2), and a weaker action of these muscles is involved in the formation of the trough-like 'preparatory' stage. When in addition the tongue is elongated a groove forms along its lateral margins (Pl. 1, fig. 3), due to the strong action of the transverse musculature, which, by reducing the transverse diameter at a time when the vertical diameter is already restricted by the genio- and hyoglossi, forces the tongue to elongate in the longitudinal direction.

The twisting of the tongue found in the 'throwing' and 'guarding' stages is brought about chiefly by the unilateral action of the styloglossus, the left muscle throwing the ingesta between the right grinding teeth. Twisting the tongue brings the margin under which the appropriate muscles lie into strong prominence (Pl. 1, fig. 4). The tip of the tongue can be twisted independently of the main body (Pl. 1, fig. 5), probably by unilateral action of the inferior longitudinal muscle, but this cannot be determined by palpation.

The pressure of the tip of the tongue on the palate in the early stages of deglutition may be assisted by the lingual cap, a thickening of the lamina propria of the mucous membrane near the tip of the tongue (Abd-el-Malek, 1939). Contraction of the intrinsic muscles of the tongue within the cap causes it to stand out on the surface and become sharply defined from the remainder of the tongue, particularly on the dorsum (Pl. 1, fig. 6). When the tip is made firm in this manner it forms a relatively rigid mass that can be pressed against the teeth or palate by other muscles. The cap is maintained in its firmed condition as the ingesta are forced to move by pressure. The longitudinal muscles working from the firmed cap as an origin may help to pull the back of the tongue anteriorly in the stage of 'slide preparation'.

#### CLINICAL OBSERVATIONS

In patients suffering from tongue injuries such as severe wounds or painful scars, or after operations on the tongue involving the removal of half its substance, a stomach tube is essential for feeding. In normal conditions it is equally easy to masticate on either side, but in cases of hemiplegia of the right side, the patient cannot, at the beginning of his illness, masticate on the left unparalysed side though the mandibular muscles on that side are still healthy and active. This is because he cannot throw the ingesta between the left grinding teeth, owing to the impaired



function of the muscles of the right half of the tongue. This explains why these patients, suffering from right hemiplegia, cannot avoid accumulation of food on the right side of the vestibule of the mouth. They feel discomfort as they are unable to masticate the food on the healthy side unless they place it between the teeth by hand, and even then after the first crushing movement it spreads between the right teeth. The bite on the paralysed side is weak as it has to be carried out by the muscles of mastication of the opposite side.

In cases of epithelioma of the tongue, if the jaws are left intact, the patient can, after recovery from operation, only masticate on the affected side, for it is only to this side that his tongue can throw the food, though he can bite on the healthy side normally. Swallowing also may be difficult, the bolus escaping to the paralysed side where it may be retained in the pyriform area.

#### SUMMARY

1. The action of the tongue in mastication has been observed in subjects who have lost some of their teeth.

2. The tongue takes part in all stages, defined as these of 'preparation' when the tongue becomes trough-like, 'throwing' the food between the teeth, 'guarding' the food from falling back from the teeth, 'sorting out' the particles and 'bolus formation', and in swallowing.

3. The muscles of the right side of the tongue throw the food between the left grinding teeth and prevent it from escaping during chewing movements, and this explains why, in hemiplegia the patient is forced to chew on the paralysed side.

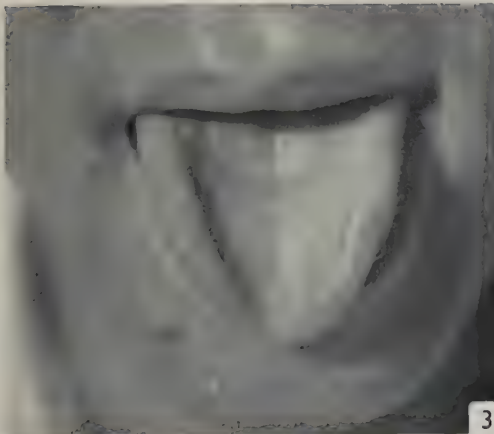
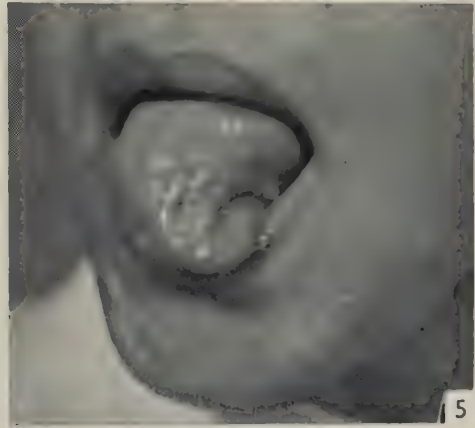
I wish to express my thanks to Prof. R. W. Haines and Prof. H. A. Harris for their help and criticism.

#### REFERENCES

- ABD-EL-MALEK, S. (1939). Observations on the morphology of the human tongue. *J. Anat., Lond.*, **73**, 201-210.
- GIBBONS, J. W. (1898). The human tongue in mastication and swallowing. *Brit. J. dent. Sci.* **41**, 869-879.
- HARRIS, P. (1927). X-ray study of movements of the tongue. *Laryngoscope, St Louis*, **37**, 235-239.
- JOHNSTONE, A. S. (1942). A radiological study of deglutition. *J. Anat., Lond.*, **77**, 97.
- WHILLIS, J. (1946). Movements of the tongue in swallowing. *J. Anat., Lond.*, **80**, 115-116.

#### EXPLANATION OF PLATE 1

- Fig. 1. The tongue with depressed centre and raised margins, as in preparation for the reception of food.
- Fig. 2. Longitudinal ridges due to strong action of the superior longitudinal muscles.
- Fig. 3. Lateral grooves due to the action of the transverse musculature.
- Fig. 4. Twisting of the tongue by the unilateral action of the styloglossus.
- Fig. 5. Independent twisting and down-turning of the tip of the tongue.
- Fig. 6. The lingual cap, with its concave posterior margin, is outlined on the dorsum of the tongue.



ABD EL-MALEK—THE PART PLAYED BY THE TONGUE IN MASTICATION AND DEGLUTITION

(Facing p. 254)





## IN MEMORIAM

FREDERIC WOOD JONES, D.Sc., F.R.C.S., F.R.S., 1879-1954

## AN APPRECIATION\*

I first met Wood Jones in 1913 at a meeting of the Anatomical Society, when I was still a medical student. I do not recollect much of the general proceedings of that meeting, but I very well remember Wood Jones. I remember him particularly because, although at that time he was one of the younger members of the Society, he contributed so conspicuously to the interest and liveliness of the meeting by the pertinence and originality of the comments which he offered to discussions on a great variety of subjects. Obviously, he was a man of an unusually alert and inquiring mind, with a remarkable width of knowledge on matters anatomical and anthropological. I met him on several occasions during the next two or three years, sometimes visiting him in his laboratory to seek answers to questions of comparative anatomy which at the time interested me, and sometimes to attend the stimulating lectures which he gave at the Royal College of Surgeons. Already during my student days Wood Jones had made a number of important observations which were beginning to be incorporated in current anatomical teaching (such as those dealing with the so-called 'sulcus subclaviae' of the first rib, the real nature of the meningeal grooves on the parietal bone, and the functional history of the coelom and diaphragm). All these papers were 'exciting' because they presented new interpretations and novel points of view, and because they made one realize that quite a number of the statements to be found in the standard text-books of anatomy were by no means to be accepted as final. I remember, also, that in the *Lancet* in 1913 he wrote what seemed to me to be a rather important article on the lesions produced by judicial hanging, for this included recommendations regarding the proper adjustment of the rope in order to ensure instantaneous death.

But, even before he engaged in these anatomical studies, Wood Jones had already had wide and varied experiences in field work in different parts of the world. For example, he had spent a year in Nubia with Elliot Smith undertaking field work in anthropology for the Egyptian Government Archaeological Survey, and the results of his studies (concerning a variety of subjects such as the mode of burial from the Predynastic Period to the Ptolemaic Period, and the sexual variations and pathological lesions in the skeletal material examined) were recorded in two of the Bulletins published by the Survey. Still earlier, he had spent over a year on the Cocos-Keeling Islands, where he occupied the position of Medical Officer to the Eastern Extension Telegraph Company. During this comparatively short period (and in addition to his medical duties) he accumulated a great number of original observations on corals and on the local flora and fauna. Some of these observations were published as separate papers in scientific journals, but most of them were collected in a book *Coral and Atolls* which appeared in 1910, and on the basis of which Wood Jones received the D.Sc. degree of London University. Now, I had had a brief

\* Grateful acknowledgment is made to Dr Archer Pearson of Poynton, Cheshire, for the photograph of Professor Wood Jones which is reproduced in the plate.

acquaintance with this book as early as 1912, but it was not until about three years ago (when I had the opportunity of staying for a few days on a little coral island at the southern end of the Great Barrier Reef) that I studied it in detail and came to recognize what a remarkable book it is. It is a remarkable book because it embodies the results of a most illuminating study of coral growth and atoll formation by a man who had only obtained his medical qualification a year or two previously, who had had no previous training in zoology beyond that provided during the first year of the ordinary medical curriculum, and who had actually completed all the field work on which his conclusions were based while still in his twenties. The book met with a somewhat unfavourable reception when it was first published, mainly because the conclusions ran counter to the rather firmly held opinions of one of the reviewers. But I have been informed by Professor C. M. Yonge, F.R.S., Leader of the Great Barrier Reef Expedition of 1928-29, that when the members of this expedition started work it was to Wood Jones's book that they turned for the best account then available of a living coral reef, and that even where they disagreed it made them think. This latter comment, incidentally, applies characteristically to several of Wood Jones's publications in other fields of study for, although his conclusions by no means always met with acceptance, his lines of argument were always fertile in the discussions which they provoked among his contemporaries. As a matter of fact, I suspect that he sometimes quite deliberately overstated his conclusions just in order, by being provocative, to compel a reconsideration of commonly held views which he believed to be seriously in need of revision.

It is not possible here to attempt an evaluation of the evidence which Wood Jones assembled in his book on coral and atolls, but it may be pointed out that he was one of the first to study corals in the field as living animals and to demonstrate that some of the morphological variations which they show represent reactions to different environments and are not indicative of specific differences. He also drew attention to the importance of sedimentation and the action of currents in determining the characteristic shape of atolls, though it may be that here he somewhat over-emphasized this factor.

One very important result of Wood Jones's visit to the Cocos-Keeling Islands must be mentioned—his marriage in 1910 to Gertrude Clunies-Ross, daughter of George Clunies-Ross, who was Governor of the Islands. There followed a life-long partnership, a partnership to which he continually paid tribute by dedicating to his wife almost every one of his books published thereafter.

Wood Jones's decision, almost immediately after qualification in 1904, to join the Eastern Extension Telegraph Company disappointed a number of his anatomical colleagues at the time, for even as a medical student at the London Hospital he had contributed original observations to the *Journal of Anatomy and Physiology* (as it was then called) and had been marked down by his teacher, Arthur Keith, for an academic career. But he was essentially a wanderer and a seeker after adventure, and his subsequent career showed clearly that his major interests were in field work rather than laboratory work, and in that aspect of general zoological study which is sometimes included under the comprehensive term 'natural history'. Although he was best known as a comparative anatomist, his focus of attention always seemed to be more on living creatures than on academic morphology.



FREDERIC WOOD JONES

*(Facing p. 256)*





When Wood Jones returned from Nubia in 1908, he went to Manchester University to be Lecturer in Anatomy with Elliot Smith. In 1910 he went to St Thomas's Hospital, and in 1912 was appointed Lecturer in Anatomy at the London School of Medicine for Women (a position which was elevated to a professorship in 1914). During these few years he wrote a series of interesting papers on the comparative anatomy of the genitalia, the results of which were summarized in an Arris and Gale Lecture at the Royal College of Surgeons in 1915 on 'The Influence of the Arboreal Habit in the Evolution of the Reproductive System'. In the first World War, he took a commission in the R.A.M.C. and was stationed at the Military Orthopaedic Hospital at Shepherd's Bush. His war work resulted in two main publications, a paper on voluntary muscular movements in cases of nerve lesions (in which he demonstrated the great variety of 'trick movements' whereby patients with partial motor paralysis are able to effect movements normally determined by the paralysed muscles), and a book on *The Principles of Anatomy as seen in the Hand* which was based on a series of lectures given as part of a course of instruction to R.A.M.C. officers. This book provided a most stimulating introduction to the study of the whole subject of human anatomy and, like myself, there must have been many who derived inspiration for their future careers from the way in which the principles of anatomy were presented to the reader. In 1916 there appeared another book, *Arboreal Man*, which as an introductory study may justly be regarded as a little 'classic' on the comparative anatomy of man and the other Primates. Even to-day it can be read with profit; indeed, I still find myself taking it down from the shelf to peruse from time to time for the pleasure of enjoying the freshness of the style and the originality of approach. Wood Jones had a facile pen and always presented his arguments in a most attractive and persuasive form, and it is true to say, I think, that almost everything he wrote contained some novel idea or some new point of view which inevitably stimulated productive trains of thought. In the years 1918 to 1920 he continued to publish papers on comparative anatomy and developed his well-known thesis that man is distinguished by the retention of a number of anatomical characters which are more primitive than the equivalent characters in other Primates. He argued from this that the phylogenetic sequence which ultimately led to man must have had a very long period of evolutionary independence, and he also argued along the same lines that man has a closer relationship to the aberrant Primate *Tarsius* than to any of the other groups of Primates. This thesis finally led to a lively symposium at a meeting of the Zoological Society in 1919, when some of his conclusions were rather severely criticized.

At the end of 1919 Wood Jones went to Australia to occupy the Chair of Anatomy at Adelaide University, where he remained for eight years. These were busy years of expeditions and field studies, during which he made a number of journeys to the northern part of South Australia, and his intense enjoyment of the opportunities for travel were made very apparent to me at the time in the occasional letters I received from him while I myself was experiencing the excitements of travel in Borneo. In the course of his expeditions extensive zoological, botanical and anthropological collections were made, some of which led to the discovery of several new marsupial species as well as many new species of invertebrates. At this time also, he published a revision of the South Australian Jerboa mice and a series of short papers

on the external characters of pouch embryos of marsupials, and between 1923 and 1925 he was responsible for a systematic catalogue of the mammals of South Australia. This last is perhaps one of Wood Jones's best works on comparative anatomy—it was certainly his most complete and systematic study. Indeed, it is probably true to say that, of all his varied contributions to comparative anatomy, his work on marsupials is likely to be the most enduring. In 1927 there was a brief interlude from Australia when Wood Jones filled the Rockefeller Chair of Anthropology in the University of Hawaii for two years. Here he began a study of the 'non-metrical' morphological characters of the human skull in different races, and also prepared a useful hand-book on *Measurements and Landmarks in Physical Anthropology*. In 1929 he published in book form, under the title *Man's Place among the Mammals*, observations and notes which he had been accumulating for many years previously. As with all his publications, this book was almost entirely illustrated by the author. Wood Jones was remarkably proficient as an anatomical artist, and the style of his line drawings was not only aesthetically pleasing—they were also notable for their accuracy and clarity.

In January 1930, Wood Jones returned once more to Australia, this time to occupy the Chair of Anatomy at the University of Melbourne. During the next few years he made several visits to the semi-arid 'mallee country' in the northern part of the State of Victoria, as well as to many islands of the Bass Strait. But in spite of all this field work, he still found time for a number of papers on strictly anatomical subjects. One of the most interesting of these was based on his re-examination of the skeletal characters of the fossil marsupial which had been described thirty years previously by Baldwin Spencer and named by him *Wynyardia*. Wood Jones demonstrated by careful comparisons that this extinct genus is closely allied to *Trichosurus* and is in no sense the annectant form between the Polyprotodontia and the Diprotodontia as some had supposed. The remarkable width of Wood Jones's interests was further shown during his years in Australia by his contributions to ornithology. Thus, he collected data during his sea voyages on the flight of sea-birds, published notes on the breeding habits of birds on the islands off the coast of Victoria, and also made an anatomical study of the unusually well-developed olfactory apparatus in the Tubinares. These ornithological studies, which are scattered in a number of Australian journals, bear witness to his aptitude for field work, and I think it may be said that he never undertook any journey without bringing back natural history 'spoils' of interest and importance.

At the end of 1937, Wood Jones returned to England, leaving behind him in Australia a reputation which will endure for very many years. His abilities were recognized by the conferment of honorary degrees, as well as by the numerous invitations which he received to deliver memorial lectures and orations. As further evidence of the esteem in which he was held, a full-length oil painting of him now hangs in the entrance hall of the Australasian College of Surgeons in Melbourne. On his return to England, he went to Manchester to occupy the Chair of Anatomy there for seven years. During that time he continued his anatomical work uninterruptedly, and published several papers dealing with matters of human and comparative anatomy, and he also undertook the arduous task of editing the seventh edition of *Buchanan's Manual of Anatomy*. In 1944 he published his *Structure and*



*Function as seen in the Foot*, in some ways a companion to his earlier work on the hand but of more particular importance to orthopaedic surgeons. Like many of his works, this book is enlivened by numerous references to the writings of old anatomists (during his long life he had collected a most valuable library of old anatomical works, and his knowledge of the history of anatomical discovery was quite unusual). In 1948, Wood Jones assumed his last academic office, that of the Sir William Collins Professor of Human and Comparative Anatomy at the Royal College of Surgeons and Conservator of the Museum. Here he settled down to reorganize and rebuild the famous Hunterian Collection which had been so seriously depleted by the disastrous bombing during the War, and to continue his lectures and writings.

Towards the latter part of his career, Wood Jones developed in much more detail his personal convictions (which had become increasingly evident in his previous writings) regarding some of the general problems of evolution. For example, in a series of books, *Design and Purpose* (1942), *Habit and Heritage* (1943), *Hallmarks of Humanity* (1948) and *Trends of Life* (1953), he affirmed his adherence to a somewhat modified Lamarckian interpretation of evolution and at the same time expressed himself as an uncompromising 'anti-Darwinian'. He also continued to argue vigorously against any close phylogenetic relationship between man and other Primates (with the solitary exception of *Tarsius* which he still held to be the only surviving representative of the ancestral stock from which man was originally derived as far back as Eocene times). I cannot help thinking that here he carried his arguments too far and sometimes rather seriously overstated the evidence on which he relied, and his phraseology, as well as his needlessly caustic disparagement of some of the great biologists of past days, also give me the impression (with which, perhaps, not all will agree) that he had developed a rather strongly emotional attitude towards the whole conception of evolution. But, in spite of this seemingly biased approach, his controversial books and essays were always well worth reading, for they contained a great deal of new or little known information, and it was impossible not to admire the dialectical skill with which he marshalled his arguments—even if it appeared that his evidence was sometimes presented with an almost deceptive ingenuity. He was obviously impressed with the precision and detail of functional adaptations in the animal world, as presumably all zoologists are. But, unlike zoologists in general, he allowed himself to be so overwhelmed with this outstanding characteristic of living organisms that he was content to ascribe all adaptations to an obscure and undefined kind of directive force, and simply to leave it at that. There was, indeed, a distinct element of mysticism in his attitude to some of the fundamental problems of organic life and, as a result, his more popular writings aroused considerable response from the class of general reader to whom the methods and philosophy of science make little appeal.

There is no doubt that Wood Jones's wide reputation as an anatomist gained much from his skill as a writer, as also from his astuteness in controversy. Indeed, there can have been few anatomists whose writings were more widely read. As already noted, he wrote with great ease and fluency, he had a most attractive literary style, and he was remarkably persuasive in the formulation of his arguments. Many of his lectures and orations were collected in book form, and he also wrote delightful essays on general subjects which he published in separate articles and in two books,

*Unscientific Essays* (1924) and *Unscientific Excursions* (1934). These two volumes of essays are mostly based on apparent trivialities culled from the many observations and experiences of his extensive travels. But each one of them contains some original point of view or some novel idea which intrigues the reader and stimulates a train of thought. They also bear witness to the author's wide range of interests, and to his capacity for seeing so many apparently ordinary things from a slightly unusual angle and thereby rendering them to that extent extraordinary.

It is a rather remarkable fact that, although Wood Jones never occupied a position in any University for more than a few years, he always seems to have left behind him an abiding tradition (almost, one might say, a 'legendary' tradition) of his outstanding and vigorous personality. And, although he only occupied the Chair of Anatomy at Manchester University for seven years, he made such a strong impression on the student body that they subscribed to institute an annual 'Wood Jones Lecture'—surely a most unusual tribute after so short a tenure of office. Incidentally, Wood Jones himself gave the first of these lectures in 1946.

That Wood Jones during his academic career was able to complete such an extensive list of publications (he was the author of fifteen books as well as of very numerous scientific papers, lectures and articles) seems surprising in view of his many activities in the field and his academic responsibilities. He was indeed a man of great mental energy and restless curiosity; he was also a man of strong opinions and sometimes gave an appearance of intolerance towards those who disagreed with him. He tended to be very critical of the development of new technical methods in anatomical research, even going so far as to imply that any method but that of scalpel and forceps (and perhaps the low power of the microscope) was beyond the proper domain of the science of anatomy. It is little wonder, therefore, that he was out of sympathy with many of the younger generation of anatomists; so much so, indeed, that he occasionally allowed himself to be almost too brusque in his attitude towards them and their work. But although he was possessed of a certain natural acerbity of temperament which impelled him to express his views in somewhat unaccommodating terms, Wood Jones certainly contributed his full share (and more) to the store of comparative anatomical knowledge, and the stimulus which he gave to his students and contemporaries by his lectures and writings can hardly be exaggerated.

Frederic Wood Jones died on 29 September 1954, at the age of 75, enduring his last illness with the greatest fortitude and courage.

W. E. LE GROS CLARK

#### PROFESSOR F. WOOD JONES'S BIBLIOGRAPHY\*

1900. A note on an outgrowth of a lymphoid nature from the junction of the large and small intestine of a frog (*Rana temporaria*). *Proc. Anat. Soc.*, in *J. Anat., Lond.*, **34**, xlv-xlvi.
1901. (With Arthur Keith.) A note on the development of the fundus of the human stomach. *Proc. Anat. Soc.*, in *J. Anat., Lond.*, **36**, xxxiv-xxxviii.
1902. The musculature of the bladder and urethra. *Proc. Anat. Soc.*, in *J. Anat., Lond.*, **36**, li-lvi.
1903. The reconstruction of an early human embryo. *Proc. Anat. Soc.*, in *J. Anat., Lond.*, **38**, lxviii-lxx.

\* Compiled by Miss Jessie Dobson, B.A., M.Sc., and Professor F. Goldby.

1904. The nature of the malformations of the rectum and urogenital passages. *Brit. med. J.* **2**, 1630-1634.
1905. A visit to a wild beast show. *Lond. Hosp. Gaz.* **11**, 204-206.
1905. A letter, somewhat medical. *Lond. Hosp. Gaz.* **12**, 59-63.
1907. On the growth-forms and supposed species in corals. *Proc. zool. Soc. Lond.* pp. 518-556.
1907. A large Ribbon Fish. *Field*, **110**, 147.
1907. Some Coral Island Fish. *Badminton Magazine*, pp. 489-500. November.
1908. *On the Rate of Growth of the Reef-building Corals*, pp. 15. London: John Bale, Sons and Daniellson.
1908. (With G. Elliot Smith.) Bulletin no. I: *The Egyptian Government Archaeological Survey of Nubia*; and supplementary report. Cairo: National Printing Office.
1908. The post-mortem staining of bone produced by the ante-mortem shedding of blood. *Brit. med. J.* **1**, 734-736.
1908. The examination of the bodies of one hundred men executed in Nubia in Roman times. *Brit. med. J.* **1**, 736-737.
1908. (With G. Elliot Smith.) Bulletin no. II: *The Egyptian Government Archaeological Survey of Nubia*. Cairo: National Printing Office.
1908. Some lessons from ancient fractures. *Brit. med. J.* **2**, 455-458.
1908. Nature's colonists. *Lond. Hosp. Gaz.* **15**, 69-72; 103-107; 120-130.
1909. The skin lesions caused by the Milleporae. *Brit. med. J.* **1**, 659.
1909. The fauna of the Cocos-Keeling Atoll. *Proc. zool. Soc. Lond.* pp. 132-160.
1909. On a theory of atoll formation: the coral island question. *Proc. zool. Soc. Lond.* pp. 671-679.
1910. The development and malformations of the glans and prepuce. *Brit. med. J.* **1**, 137-138.
1910. The building of atolls. *Zool. Anz.* **35**, 399-404.
1910. On the real significance of the 'sulcus subclaviae' B.N.A. and the markings on the first rib. *Anat. Anz.* **36**, 25-28.
1910. An anatomical inquiry into the pathway of tubercular infection. *Lancet*, **1**, 914-916.
1910. *Coral and Atolls*, pp. xxiii+392. London: Lovell Reeve.
1910. On the relation of the limb plexuses to the ribs and vertebral column. *J. Anat., Lond.*, **44**, 377-393.
1910. (With G. Elliot Smith.) *The Archaeological Survey of Nubia*. Report for 1907-1908. Vol. II. Report on the human remains. Cairo: National Printing Department.
1910. The coral island question: a discussion carried on in *Nature*. *Nature, Lond.*, **84**, 528-529; **85**, 106; 139.
1911. Johannis Browne; a byegone anatomist of St Thomas's. *St Thom. Hosp. Gaz.* **21**, 55.
1911. The delimitation of the rectum and its subdivisions; an anatomical introduction to a discussion on the operative treatment of cancer of the rectum. *Proc. R. Soc. Med.* **4** (Surg. Sect.), 85-98.
1911. Thomas Wharton: a byegone anatomist of St Thomas's. *St Thom. Hosp. Gaz.* **21**, 108-112.
1911. Variations of the first rib associated with changes in the constitution of the brachial plexus. *J. Anat., Lond.*, **45**, 249-255.
1911. William Cheselden; a byegone anatomist of St Thomas's. *St Thom. Hosp. Gaz.* **21**, 170.
1912. Some markings on the cervical vertebrae. *J. Anat., Lond.*, **46**, 41-44.
1912. Extroversion of the bladder and some problems in connection with it. *J. Anat., Lond.*, **46**, 193-210.
1912. On the grooves on the ossa parietalia commonly said to be caused by the arteria meningea media. *J. Anat., Lond.*, **46**, 228-238.
1912. The vascular lesion in some cases of middle meningeal haemorrhage. *Lancet*, **2**, 7-12.
1912. Nubas, ancient and modern. (Abstract.) *Rep. Brit. Ass.*, p. 619.
1912. The lesions caused by judicial hanging; an anthropological study. (Abstract.) *Rep. Brit. Ass.*, pp. 619-620.
1912. Some nerve markings on the lumbar vertebrae. *J. Anat., Lond.*, **47**, 118-120.
1912. The radiographic diagnosis of rudimentary ribs. *Lond. Hosp. Gaz.* **18**, 166.
1913. The ideal lesion produced by judicial hanging. *Lancet*, **1**, 53.
1913. The story of the diaphragm. (Abstract.) *St Thom. Hosp. Gaz.* **23**, 32-33.
1913. Some anatomical considerations of the disposition of the sciatic nerve and the femoral artery: with suggestions as to their clinical significance. *Lancet*, **1**, 752-753.
1913. The anatomy of cervical ribs. *Proc. R. Soc. Med.*, **6** (Clin. Sect.), 95-113.



1913. The functional history of the coelom and the diaphragm. *J. Anat., Lond.*, **47**, 282-318.
1914. Some points in the nomenclature of the external genitalia of the female. *J. Anat., Lond.*, **48**, 73-80.
1914. Some phases in the reproductive history of the female mole (*Talpa europea*). *Proc. zool. Soc. Lond.* pp. 191-216.
1914. The morphology of the external genitalia of the Mammals. (Arris and Gale Lecture.) Part I. *Lancet*, **1**, 1017-1023.
1914. The morphology of the external genitalia of the Mammals. (Arris and Gale Lecture.) Part II. *Lancet*, **1**, 1099-1103.
1914. The lower ends of the Wolffian ducts in a female Pig embryo. *J. Anat., Lond.*, **48**, 268-273.
1914. Section III. The articulations, pp. 211-311. In *Morris's Human Anatomy*, 5th ed. London: J. A. Churchill.
1915. The influence of the arboreal habit in the evolution of the reproductive system. *Lancet*, **1**, 1113-1124.
1915. The Chelonian type of genitalia. *J. Anat., Lond.*, **49**, 393-406.
1915. The homologies of the Chelonian and Mammalian types of genitalia. *J. Anat., Lond.*, **50**, 1-23.
1915. The explanation of a recto-urethral anomaly, and some points in normal anatomy. *Lancet*, **2**, 860-861.
1916. The genitalia of *Galeopithecus volans*. *J. Anat., Lond.*, **50**, 189-203.
1916. The genitalia of the Cheiroptera. Part I. Megachiroptera. Part II. Microchiroptera. *J. Anat., Lond.*, **51**, 36-60.
1916. *Arboreal Man*, pp. x+230. London: Edward Arnold.
1917. The genitalia of *Tupaia*. *J. Anat., Lond.*, **51**, 118-126.
1917. The relation of structure to function as seen in a mechanism of the venous system. *Lancet*, **1**, 574-575.
1917. The structure of the orbito-temporal region of the skull of *Lemur*. *Proc. zool. Soc. Lond.* pp. 323-329.
1918. Remarks upon specimens from The Prosectorium illustrating the effects of rickets. (Abstract.) *Proc. zool. Soc.* p. 196.
1918. (With E. Rickards.) On abnormal sexual characters in twin goats. *J. Anat., Lond.*, **52**, 265-275.
1918. *The Problem of Man's Ancestry*, pp. 48. London: Society for Promoting Christian Knowledge.
1918. The sublingua and the plica sublinguata. *J. Anat., Lond.*, **52**, 345-354.
1918. The human hand and foot. *J. inc. Soc. Masseuses*, **4**, 13-18.
1919. Chapter V: The Origin of Man, pp. 99-133. In *Animal Life and Human Progress*. Ed. Arthur Dendy. London: Constable.
1919. Part editor of *Buchanan's Manual of Anatomy*. London: Baillière, Tindall and Cox.
1919. What we know of ourselves. *J. inc. Soc. Masseuses*, **5**, 1-6.
1919. Voluntary muscular movements in cases of nerve lesions. *J. Anat., Lond.*, **54**, 41-57.
1919. Voluntary muscular movements in cases of nerve injuries. *Lancet*, **2**, 907-909.
1919. Discussion on the zoological position and affinities of *Tarsius*. *Proc. zool. Soc. Lond.*, pp. 491-494.
1920. *The Principles of Anatomy as seen in the Hand*, pp. viii+325. London: J. and A. Churchill.
1920. The anatomy of snapping hip. *J. orthop. Surg.* **2**, 1-3.
1920. Anatomy and the medical student; the history of the legalizing of anatomy. *Adelaide Med. Stud. Soc. Rev.* **11**, 22-26.
1920. The external characters of pouch embryos of Marsupials. No. 1. *Trichosurus vulpecula*, var. *typians*. *Trans. roy. Soc. Aust.* **44**, 360-373.
1921. The 'flight' of Flying Fish; with an introductory note by Sir David Wilson-Barker. *Nature, Lond.*, **107**, 233-234.
1921. A necrology of anatomists. *Adelaide Med. Stud. Soc. Rev.* **12**, 21-24.
1921. Voluntary muscular movements in cases of nerve injuries and trick movements, pp. 394-413. In *The Orthopaedic Surgery of Injuries*, Vol. II, Ed. Sir Robert Jones. London: Henry Frowde.
1921. Human and other tails. *Nature, Lond.*, **107**, 487.

1921. The external characters of pouch embryos of Marsupials. No. 2. *Notoryctes typhlops*. *Trans. roy. Soc. S. Aust.* **45**, 36–39.
1921. The status of the Dingo. *Trans. roy. Soc. S. Aust.* **45**, 254–263.
1921. On the habits of *Trichosurus vulpecula*. *J. Mammal.* **2**, 187–193.
1922. The external characters of pouch embryos of Marsupials. No. 3. *Isodon barrowensis*. *Trans. roy. Soc. S. Aust.* **46**, 39–45.
1922. The external characters of pouch embryos of Marsupials. No. 4. *Pseudochirops dahli*. *Trans. roy. Soc. S. Aust.* **46**, 119–130.
1922. The flora and fauna of Nyut's Archipelago and the Investigator Group. No. II. The Monodelphian Mammals. *Trans. roy. Soc. S. Aust.* **46**, 181–193.
1922. The rat menace. *Educ. Gaz. S. Aust.* **38**, 189–191.
1922. On the dental characters of certain Australian rats. *Proc. zool. Soc. Lond.* pp. 587–598.
1923. *The Mammals of South Australia*, pp. 131. Part I. The Monotremes and Carnivorous Marsupials. Adelaide: Government Printer.
1923. The flora and fauna of Nyut's Archipelago and the Investigator Group. No. VI. The Didelphian Mammals. *Trans. roy. Soc. S. Aust.* **47**, 82–94.
1923. *The Ancestry of Man; Man's place among the Primates*, pp. 34. (Douglas Price Memorial Lecture.) Brisbane: Gillies.
1923. The external characters of pouch embryos of Marsupials. No. 5. *Phascolarctus cinereus*. *Trans. roy. Soc. S. Aust.* **47**, 129–135.
1923. The Marsupial genus *Thalacomys*. A review of the Rabbit Bandicoots with a description of a new species. *Rec. S. Aust. Mus.* **2**, 333–352.
1923. The external characters of pouch embryos of Marsupials. No. 6. *Dasycercus cristicauda*. *Trans. roy. Soc. S. Aust.* **47**, 136–141.
1923. The external characters of pouch embryos of Marsupials. No. 7. *Myrmecobius fasciatus*. *Trans. roy. Soc. S. Aust.* **47**, 195–200.
1923. *The position of Anatomy in the modern medical curriculum and the conception of cytotecsis*, pp. 25. (Adelaide University Commemoration Address.) Adelaide: Hassall Press.
1923. A defence of the foramen of Magendie. *Lancet*, **2**, 486.
1924. Cytotecsis. A phenomenon explaining the course of embryonal development. *Lancet*, **1**, 684–685.
1924. Six hitherto undescribed skulls of Tasmanian natives (with an account of the palate and teeth by T. D. Campbell). *Rec. S. Aust. Mus.* **2**, 459–469.
1924. The flora and fauna of Nyut's Archipelago and the Investigator Group. No. XV. The Pearson Island rat and the Flinders Island Wallaby. *Trans. roy. Soc. S. Aust.* **48**, 10–14.
1924. The external characters of pouch embryos of Marsupials. No. 8. *Dendrolagus matschiei*. *Trans. roy. Soc. S. Aust.* **48**, 79–82.
1924. The status of the Kangaroo Island Kangaroo. (*Macropus fuliginosus* Desm.) *Proc. zool. Soc. Lond.* pp. 451–460.
1924. The external characters of pouch embryos of Marsupials. No. 9. *Phascolomys tasmaniensis*. *Trans. roy. Soc. S. Aust.* **48**, 145–148.
1924. *The Mammals of South Australia*, pp. 132–270. Part II. Bandicoots and Herbivorous Marsupials. Adelaide: Government Printer.
1924. The Marsupials of South Australia. Pp. 31–33 in *Handbook of Australian Association for the Advancement of Science*. Adelaide Meeting.
1924. On the specialized incisor teeth of some of the didactylous Marsupials. *Trans. roy. Soc. S. Aust.* **48**, 187–191.
1924. *Unscientific Essays*, pp. 208. London: Edward Arnold.
1924. (With T. D. Campbell.) Anthropometric observations on South Australian aborigines, with a summary of previously recorded data. *Trans. roy. Soc. S. Aust.* **48**, 303–312.
1924. On the causation of certain hair tracts. *J. Anat., Lond.*, **59**, 72–79.
1924. The hair slope in the frontal region of man. *J. Anat., Lond.*, **59**, 80–82.
1925. (With T. D. Campbell.) A contribution to the study of eoliths: some observations on the natural forces at work in the production of flaked stones on the central Australian tablelands. *J. R. anthrop. Inst.* **55**, 115–122.
1925. The ordered arrangement of stones present in certain parts of Australia. *J. R. anthrop. Inst.* **55**, 123–128.
1925. The hair pattern of a kangaroo; a study of cause and effect. *J. Mammal.* **6**, 13–17.

1925. A revision of the South Australian Jerboa mice, with the description of a new species. *Rec. S. Aust. Mus.* 3, 1-7.
1925. The eared seals of South Australia. *Rec. S. Aust. Mus.* 3, 9-16.
1925. A new South Australian dormouse opossum. *Trans. roy. Soc. S. Aust.* 49, 96-98.
1925. The mammalian toilet and some considerations arising from it. *Proc. roy. Soc. Tasm.* pp. 14-62.
1925. Muscular movement and its regulation. *Med. J. Aust.* 2, 627-631.
1925. *The Mammals of South Australia*, pp. 271-458. Part III. The Monodelphia. Adelaide: Government Printer.
1926. Lessons learned abroad. *The Register*, Adelaide, for 8, 15, 22 May and 5 June.
1926. Flinders Chase. South Australian, Adelaide. 8 June.
1926. Some observations on the flight of sea-birds. *Trans. roy. Soc. S. Aust.* 50, 36-44.
1926. Disease and individuality. (The Lister Oration.) *Med. J. Aust.* 2, 65-71.
1926. The claims of the Australian aboriginal. *Rep. Aust. Ass. Advanc. Sci.* 18, 497-519.
1927. (With E. R. Waite.) The fauna of Kangaroo Island. Nos. 2 and 3. Reptiles, Amphibians, Mammals. *Trans. roy. Soc. S. Aust.* 51, 322-329.
1927. The mid-dorsal hair whorl of man. *Amer. J. phys. Anthropol.* 77, 89-95.
1928. (With S. Porteus.) *The Matrix of the Mind*, pp. viii + 457. Honolulu: Hawaii University Press Association. English edition. London: Edward Arnold. 1929.
1929. Man and the Anthropoids. *Amer. J. phys. Anthropol.* 12, 245-252.
1929. The Tasmanian skull. *J. Anat., Lond.*, 63, 224-232.
1929. The Australian skull. *J. Anat., Lond.*, 63, 352-355.
1929. Some landmarks in the phylogeny of the Primates. *Hum. Biol.* 1, 214-228.
1929. The distinctions of the human hallux. *J. Anat., Lond.*, 63, 408-411.
1929. Measurements and landmarks in physical anthropology. Bernice P. Bishop Museum. Bulletin No. 63, pp. 67.
1929. *Man's Place among the Mammals*, pp. xi + 372. London: Edward Arnold.
1929. The cranial characters of the Papuan dog. *J. Mammal.* 10, 329-333.
1930. The Polynesian race—a question of anthropometric method. *Man*, 30, 60-64.
1930. Sinfulness and Necks. *Stead's Rev.* 67, 3-4. May.
1930. My friend in the garden. *Stead's Rev.* 67, 11-12. June.
1930. Of map journeys. *Stead's Rev.* 67, 9-10. July.
1930. The mission and the half-caste. *Stead's Rev.* 67, 23-26. September.
1930. Some physical and biological concepts in science. *J. Coll. Surg. Aust.* 3, 56-65.
1930. Of stars and Spoonbills. *Stead's Rev.* 67.
1930. Of Atoms and Life. *Melbourne Univ. Rev.*
1930. A re-examination of the skeletal characters of *Wynyardia bassiana*, an extinct Tasmanian Marsupial. *Pap. roy. Soc. Tasm.* pp. 96-115.
1931. Of Thermometers and Bumps. *Stead's Rev.* 68, February.
1931. Of remarkable years. *Stead's Rev.* 68, 15-16. March.
1931. The non-metrical morphological characters of the skull as criteria for racial diagnosis. Part I. General discussion of the morphological characters employed in racial diagnosis. *J. Anat., Lond.*, 65, 179-195.
1931. The cranial characters of the Hawaiian dog. *J. Mammal.* 12, 39-41.
1931. The non-metrical morphological characters of the skull as criteria for racial diagnosis. Part II. The non-metrical morphological characters of the Hawaiian skull. *J. Anat., Lond.*, 65, 368-378.
1931. Of things told only to Sinbad. *Stead's Rev.* 68, 17-18.
1931. (With J. B. Turner.) A note on the sensory characters of the nipple and areola. *Med. J. Aust.* 1, 778-779.
1931. On two mandibles from Guam. *Aust. J. Dent.* 35, 243-248.
1931. The non-metrical morphological characters of the skull as criteria for racial diagnosis. Part III. The non-metrical morphological characters of the skulls of prehistoric inhabitants of Guam. *J. Anat., Lond.*, 65, 438-445.
1931. The changing point of view. (Bancroft Memorial Lecture.) *Med. J. Aust.* 2, 251-268.
1931. Of grave-yards and mound springs. *Stead's Rev.* 68, 23-24. September.
1931. On certain sciences. *Stead's Rev.* 68, 41-42. October.
1931. The external meatal notch as a primitive character. *Nature, Lond.*, 128, 458.



1932. A small-headed type of female Australian. *Man*, **32**, 38–40.
1932. The centenary of the Anatomy Act. *The Argus*, Melbourne, for 23 April.
1932. The ostrich—an apology to him and his kind. *The Argus*, Melbourne, for June 4.
1932. The mind in the making. (Wireless talks published in the *Listener In* for 7, 14, 21, 28 May and 4, 11, 18, 25 June.
1932. The Island of Tragedy and Romance (Pearson Island). Table Talk. *Christmas Annual*, pp. 12–13.
1933. The cult of Yu hi Yu; Traveller's Joy; Concerning the Dragon; Astronomers and Pansies; Paint and Petrels; In Praise of the Unnecessary; In disparagement of brains; Bobby birds and passports; Myths and Moles. A series of nine essays published in *The Argus*, Melbourne.
1933. On weight-bearing bony prominences. *Anat. Rec.* **56**, 31.
1933. The external characters of an Australian foetus. *J. Anat., Lond.*, **67**, 549–554.
1933. (With J. Wunderley.) The non-metrical morphological characters of the Tasmanian skull. *J. Anat., Lond.*, **67**, 583–595.
1933. The Chinese bandit; Medicine and magic in China; Ancient art and modern craftsman. Young China and the world. Four articles in *The Herald*, Australian Syndicated Press.
1933. The non-metrical morphological characters of the skull as criteria for racial diagnosis. Part IV. The Northern Chinese skull. *J. Anat., Lond.*, **68**, 96–108.
1934. Tasmanians and Australians. *Man*, **34**, 52–53.
1934. Contrasting types of Australian skulls. *J. Anat., Lond.*, **68**, 323–330.
1934. (With Wen-i-Chun.) The development of the external ear. *J. Anat., Lond.*, **68**, 525–533.
1934. The dorsal hair tracts of the Australian aborigine. *J. Anat., Lond.*, **69**, 91–92.
1934. *Unscientific Excursions*, pp. 207. London: Edward Arnold.
1934. *Seabirds Simplified*, pp. 48. London: Edward Arnold.
1934. Australia's vanishing race. (*The National Broadcast Lectures*), pp. 40. Sydney: Angus and Robertson.
1935. The aborigines of Victoria, pp. 136–142. In *Australian and New Zealand Association for the Advancement of Science Handbook for Victoria*.
1935. The medico-legal aspect of judicial hanging. *Proc. med.-leg. Soc. Vict.* 1931–33, pp. 1–7.
1935. *Tasmania's Vanished Race*, pp. 32. Sydney: Australian Broadcasting Commission.
1935. The Master Surgeon. (The George Adlington Syme Oration) *Aust. N.Z. J. Surg.* **5**, 34–47.
1935. The aborigines of Australia, pp. 13. In B.M.A. Handbook. *The Book of Melbourne*.
1935. Unity in Nature. *J. Instn. Engrs. Aust.* **7**.
1935. *The Problems of Life: (1) Mechanism and Vitalism; (2) Life and Growth; (3) Living and Non-living.* (The Livingstone Lectures.) Sydney: Camden College.
1936. Skulls from the Purari Plateau, New Guinea. *J. Anat., Lond.*, **70**, 405–409.
1936. The breeding of the Sooty Shearwater (*Puffinus griseus*) on Tasman Island. *S. Aust. Orn.* **13**, 197–200.
1936. The wanderings of the Albatross. *Emu*, **36**, 103–105.
1936. Notes on the breeding of the short-tailed Shearwater (*Puffinus tenuirostris*) in 1936. *S. Aust. Orn.* **13**, 223–228.
1936. The McCoy Society's expedition to Lady Julia Percy Island. *Nature, Lond.*, **138**, 906–908.
1936. The toll of the beaches. *Vict. Nat., Melb.*, **53**, 138–140.
1937. The breeding of Prions on the islands off the coast of Victoria. *Emu*, **36**, 186–188.
1937. The olfactory organ of the Tubinares. Part I. General introduction and anatomical account of the olfactory apparatus of *Puffinus tenuirostris*. *Emu*, **36**, 281–286.
1937. Grafton Elliot Smith; life and work of a famous Australian. *Aust. nat. Rev.* **1**, 1–8.
1937. The breeding of the White-faced Storm Petrel on South Australian Islands. *S. Aust. Orn.* **14**, 35–41.
1937. Lady Julia Percy Island. *Discovery*, **18**, 140–142.
1937. Report No. I. McCoy Society. Lady Julia Percy Island. (1) General Introduction. (2) (with A. Tubb) Aves. *Proc. roy. Soc. Vict.* **49**.
1937. The olfactory organ of the Tubinares. Part II. The development of the nasal tubes of *Puffinus tenuirostris*. *Emu*, **37**, 10–13.
1937. The derivation of the Australian fauna. (Carnegie Lecture.) *Sci. Rev. Melb. Univ. Sci. Cl.* pp. 11–16.
1937. The diving Petrel of the Bass Straits. *Vict. Nat., Melb.*, 50–55.

1937. The spirit of adventure. *Med. J. Aust.* 2, 506-512.
1937. The olfactory organ of the Tubinares. Part III. *Diomedea cauta*. *Emu*, 37, 128-131.
1937. The question of species, with particular references to the Tubinares. *Emu*, 37, 121-127.
1937. The legalization of the study of human anatomy. *Proc. med.-leg. Soc. Vict.* 2, 135-144.
1938. The external characters of a second Australian foetus. *J. Anat., Lond.*, 72, 301-304.
1938. Chapter V: 'In Egypt and Nubia', pp. 139-150. In *Sir Grafton Elliot Smith*. Ed. W. R. Dawson. London: Jonathan Cape.
1938. The cervical vertebrae of the Australian native. *J. Anat., Lond.*, 72, 411-415.
1938. The fate of the human premaxilla. *J. Anat., Lond.*, 72, 462.
1938. Reports of the McCoy Society. No. 2. Sir Joseph Banks Islands. Part 1. *Proc. roy. Soc. Vict.* 50, Aves. Section 13, 399-413.
1938. The so-called maxillary antrum of the Gorilla. *J. Anat., Lond.*, 73, 116-119.
1938. Grains and scruples. A series of essays written under the pseudonym 'An Anatomist'. *Lancet*, 2, 1325-1326, 1377-1378, 1432-1434, 1487-1488, 1541-1542.
1938. The 'thumb' of the Giant Panda. *Nature, Lond.*, 143, 157, 246.
1939. Chapter VI, pp. 72-109. In *Doctors in Shirt Sleeves*. Ed. Sir Henry Bashford. London: Kegan Paul, Trench, Trubner.
1939. The forearm and manus of the Giant Panda *Ailuropoda melanoleuca* with an account of the mechanism of its grasp. *Proc. zool. Soc. Lond.* 109, 113-129.
1939. The anterior superior alveolar nerves and vessels. *J. Anat., Lond.*, 73, 583-591.
1939. *Life and Living*, pp. 268. London: Kegan Paul, Trench, Trubner.
1939. (With V. F. Lambert.) The occurrence of the lemurine form of the ectotympanic in a primitive marsupial. *J. Anat., Lond.*, 74, 72-75.
1940. The nature of the soft palate. *J. Anat., Lond.*, 74, 147-170.
1940. The white-breasted Petrel of Southern Australia. *Emu*, 39, 277-278.
1940. The attainment of the upright posture in Man. *Nature, Lond.*, 146, 26-27.
1941. Tension lines, cleavage lines and hair tracts in Man. *J. Anat., Lond.*, 75, 248-250.
1941. The external characters of a neonatal. *Proc. zool. Soc. Lond.* 110, 199-206.
1941. The musculature of the body cavities. *J. Chart. Soc. Massage med. Gymn.* 27, 54-58.
1942. In England Now. A series of articles. *Lancet*, 1, 87, 180, 332, 570.
1942. *The Principles of Anatomy as seen in the Hand*, 2nd ed. pp. xii+418. London: Baillière, Tindall and Cox.
1942. *Design and Purpose*. (The Purser Lecture.) pp. 84. London: Kegan Paul, Trench, Trubner.
1942. Lessons from the louse—a study in symbiosis. *Lancet*, 1, 661-662.
1943. *Habit and Heritage*, pp. 100. London: Kegan Paul, Trench, Trubner.
1943. John Gilbert, ornithologist. *Nature, Lond.*, 152, 286.
1944. *Structure and Function as seen in the Foot*, pp. 329. London: Baillière, Tindall and Cox.
1944. The talocalcaneal articulation. *Lancet*, 2, 241-242.
1944. Some curiosities of mammalian reproduction. Part I. Mammals that have triumphed over anatomical handicaps. (Lloyd Roberts Lecture.) *J. Obstet. Gynaec., Brit. Emp.*, 51, 416-437.
1944. Some curiosities of mammalian reproduction. Part II. Concerning the life of the sperm. (Lloyd Roberts Lecture.) *J. Obstet. Gynaec., Brit. Emp.*, 51, 553-564.
1945. The mammalian toilet. *Proc. roy. Instn. G.B.* 33, 276-288.
1945. Some curiosities of mammalian reproduction. Part III. Mammals that produce uniovular litters. *J. Obstet. Gynaec., Brit. Emp.*, 52, 55-70.
1945. Time and Lamarck. (Lamarck Bicentenary Address.) *Proc. Linn. Soc. Lond.* part 1, pp. 11-14, 20.
1945. Science and the medical man. *Manchr med. Sch. Gaz.* 24, 36-40.
1946. Needs and their satisfaction. (Inaugural Wood Jones Lecture.) *Manchr med. Sch. Gaz.* 25, 3-11.
1946. The evolution of the foot. *Chiropodist*, 1, 5-13.
1946. Editor of *Buchanan's Manual of Anatomy*, 7th ed. London: Baillière, Tindall and Cox.
1947. The premaxilla and the ancestry of Man. *Nature, Lond.*, 159, 439.
1948. What is gout? *Lancet*, 1, 165-167.
1948. *The Hallmarks of Mankind*, pp. 85. London: Baillière, Tindall and Cox.
1948. The emergence of Man. *Chiropodist*, 3, 166-173.
1948. Present state of our knowledge of the anatomy of the Primates. *Brit. med. J.* 2, 629-631.

1949. John Hunter and his museum. *Ann. R. Coll. Surg.* **4**, 337-341.
1949. Discussion on human maxilla. *Man*, **49**, 12, 23, 35.
1949. Some proportional weights and measurements of the human body. *Ann. R. Coll. Surg.* **5**, 32-33.
1949. The evolution of the giraffe. *Nature, Lond.*, **164**, 323.
1949. The study of a generalized Marsupial (*Dasyercus cristicauda* Krefft). *Trans. zool. Soc. Lond.*, **26**, 409-501.
1950. Freedom of thought in science. (The Sir John Struthers Lecture.) *Edinb. med. J.* **56**, 509-529.
1950. Review of Sir Arthur Keith's 'Autobiography'. *Manchester Guardian* for 9 March.
1950. Classical gout. (The Heberden Oration.) *Brit. med. J.* **1**, 561-567.
1950. Ernest Whitnall; obituary notice. *J. Anat., Lond.*, **84**, 395.
1951. The human jaws and dentition. (Northcroft Memorial Lecture.) *Dent. Rec.* **71**, 1-10.
1951. The external characters of a foetal Tarsier. *Proc. zool. Soc. Lond.* **120**, 723-730.
1951. A contribution to the history and anatomy of Père David's Deer (*Elaphurus davidianus*). Ed. and part author. *Proc. zool. Soc. Lond.* **121**, 319-370.
1951. John Hunter's unwritten book. (St George's Hospital Commemoration Address.) *Lancet*, **2**, 778-780.
1951. John Hunter and the Medical Student. *St Geo. Hosp. Gaz.* **37**, 53-60.
1952. The mammalian mandible. *Proc. Anat. Soc.*, in *J. Anat., Lond.*, **86**, 489.
1952. The superman. *Brit. med. Stud. J.* **6**, 6-7.
1952. Fifty years of fallacies. *Lond. Hosp. Gaz.* **55**, 117-121.
1952. The mandible and its relation to the otocyst and auditory organ. *J. Laryng.* **16**, 473-479.
1953. John Hunter as a Geologist. *Ann. R. Coll. Surg.* **12**, 219-245.
1953. *Trends of Life*, pp. 191. London: Edward Arnold.
1953. Some readaptations of the mammalian pes in response to arboreal habits. *Proc. zool. Soc. Lond.* **123**, 33-41.
1953. Concerning nettles. *Med. Bull.* pp. 27-29. May and Baker Ltd.
1954. In praise of redness. *Med. Bull.* pp. 12-14. May and Baker Ltd.
1954. Vitamins and their kind. *Med. Bull.* pp. 52-55. May and Baker Ltd.
1954. Pigeons. *Med. Bull.* pp. 103-105. May and Baker Ltd.
1954. On gravity. *Med. Bull.* pp. 145-146. May and Baker Ltd.

The last five articles are published under the pseudonym 'Natterjack'.



## REVIEWS

*Anatomy of the Bronchial Tree.* By R. C. BROCK, 2nd ed. (Pp. viii+243; 157 illustrations; 6 × 9½ in.; 45s.) London, New York, Toronto: Oxford University Press.

*Anatomy of the Bronchovascular System.* By G. L. BIRNBAUM. (Pp. xv+300; 99 illustrations+27 plates, 7¼ × 10¼ in.; \$15.00 or 112s.) Chicago: The Year Book Publishers.

The appearance of the second edition of Mr Brock's well-known and widely appreciated book has been delayed until the recommendations of the Nomenclature Committee of the International Congress of Otolaryngology (London, July 1949; *Thorax*, 1950, 5, 222) had become generally accepted. His new chapter II describes the basis of the common nomenclature and the reasons for anatomical conformity. Does one prefer 'superior' to 'apical'? and if so will one object to 'sub-superior' only on grounds of euphony? The reluctance to abandon well-used 'cardiac' results in the compromise 'medial (cardiac)'. One cannot help a terminological smile on reading that the proposal 'lower division' for 'lingular' was defeated by eleven votes, but that 'lower division' should be appended in brackets. The International Committee had finished their session, and several members had left when it was realized that no name had been agreed upon for that portion of the right bronchus between the upper lobe and middle lobe branches; eventually the Thoracic Society decided to call it 'the lower part of the right main bronchus' and hoped that international blessing would be given. The extensive revision of the text and the remaking of numerous blocks is an earnest of the author's recognition of the need for universal acceptance of an international nomenclature. Otherwise there is little alteration in the text; new material includes the more recent work of Foster-Carter and of Boyden and his associates on variations and abnormalities in broncho-vascular pattern. The author does not, however, find the 'almost bewildering array of orifices' depicted by Appleton (1946) from bronchoscopic observations, and his experience does not support Appleton's interpretation.

Dr Birnbaum's book is a natural result of the recent major advances in the surgery of segmental resection of the lung, which needed the development of the segmental anatomy of the lungs to make it a practical procedure, and in the operative attack on the great vessels and inside the heart. The early chapters are concerned with the embryology, descriptive anatomy and anomalies of the bronchovascular system. Many of the paragraphs are quotations taken from standard text-books of embryology or anatomy with numerous additional extracts from original papers. The description of the bronchovascular system follows closely the observations of Boyden and his associates, and uses Boyden's modifications of Jackson and Huber's terminology. The second half of the book is concerned with the surgery of the pulmonary vessels, the trachea and bronchi, and excision of pulmonary tissue, and is freely illustrated by descriptions of clinical material. Of particular interest are the sections, with their useful bibliography, on experimental vascular and pulmonary surgery. It is these sections of the book which will be of greatest value, since the descriptive anatomy is so readily available from standard sources.

R. J. HARRISON

## THE BEHAVIOUR OF AUTOGRAFTS AND HOMOGRAFTS OF VAGINAL TISSUE IN RABBITS

By P. L. KROHN\*

*Department of Anatomy, University of Birmingham,  
Medical School, Edgbaston, Birmingham 15*

The potential clinical importance of successful tissue homotransplantation has made it inevitable that the factors which govern the fate of many body tissues, after they have been homografted from one individual member of a species to another, should have been studied intensively for many years. The general conditions which determine the success or failure of the graft have been most satisfactorily worked out using skin as the donor tissue (see especially Medawar, 1944, 1945). It is now generally agreed that, except in a few special circumstances, ordinary orthotopic skin homografts are treated as foreign material and are destroyed within a short time of grafting by a reaction which resembles an immunity response in many respects.

The endocrine glands represent another group of organs whose successful homografting could be of great clinical value. There is, however, much less general agreement about the fate of homografts of endocrine tissues than about the fate of skin homografts. Much of the confusion and many of the reports of success may be due to a loose interpretation of the term 'homograft' (Krohn, 1955). It is often said that endocrine homografts are more likely to survive if the host's own homologous endocrine glands have previously been removed, e.g. that grafts of ovarian tissue are more successful if the host has been spayed. Explanations of this belief have been based on the view that the host's pituitary gland becomes more active after spaying and that the extra trophic hormone, by stimulating the graft to active growth, protects it from the consequences of an immune reaction. Harris & Eakin (1949) have suggested that this process of active cell division allows tissues of the homograft either to absorb or to remove the antibodies which would be expected to develop against them. The belief that proliferation of its component cells could protect a homograft does not receive any support from the natural history of skin homografts, which, for the first few days after grafting, proliferate as rapidly as do autografts. Their phase of active growth, by multiplying the amount of potentially antigenic material, seems, on the contrary, to be a disadvantage to them rather than an advantage. For, if they remain indolent under quasi tissue culture conditions, their survival is prolonged.

Those endocrine organs which respond to the trophic principles of the pituitary are not, of course, the only organs in which rapid growth can be induced by hormones. Growth of such tissues as the components of the female reproductive tract may be stimulated by oestrogen. Experiments in which the fate of homografts of vaginal tissue has been studied in rabbits, with or without the added stimulus to

\* Now Nuffield Senior Gerontological Research Fellow.

growth of injected oestrogenic hormone, are described below and provide: (i) information about the homograft reaction to an epithelial tissue which has not previously been studied; and (ii) a test of the general hypothesis that an increased rate of growth, provided by hormonal stimulation, can prolong the life of a homograft. The behaviour of autografts of vaginal tissue is also described. Since the epithelium of the vagina of the rabbit is not uniform throughout its length (see below), it has been possible to compare the behaviour of autografts and homografts of vaginal stroma covered by two different types of epithelial cell.

#### MATERIALS AND METHODS

Adult rabbits of both sexes were used as recipients. The homografts were obtained from mature females whose breed and coat colour differed from those of the host. In two experiments (Nos. 7 and 8) grafts from two donors, one normal and one spayed, were applied at the same operation. In other experiments the grafts were removed from donors which had been previously treated with oestrogen.

Autografts were taken from both normal and oestrogen-treated rabbits.

Tables 1-3 summarize the experiments that were carried out.

#### *Structure of the vagina of the rabbit*

The rabbit's vagina is a long, distensible tube with a thick muscle coat. Its epithelial lining is not uniform in character throughout its length (Carleton, 1931). The distal third, reaching to just above the level of the opening of the urethra into the vagina (a little further cranially on the dorsal than on the ventral aspect of the vagina), is lined by squamous non-keratinizing epithelium and is generally thought to be derived embryologically from the urogenital sinus (Jost, 1943; Carr, 1953), although Baxter (1933) denies that the urogenital sinus plays any part in the formation of the vagina. The epithelium overlies a fairly thick, loose, connective tissue stroma in which there are a striking number of thin-walled blood sinuses. The remaining two-thirds of the vagina are lined by a mucus-secreting high columnar epithelium which is separated from the thick layer of underlying muscle by a thin layer of loose stromal elements. Just beneath the epithelium there is a conspicuous series of large lymphatic channels. It is generally agreed that this part of the vagina is derived from the Müllerian tissues.

#### *Methods for removing tissue for grafting*

Vaginal tissue for grafting was removed at three different levels because of the lack of uniformity of structure.

(a) *Grafts from the introitus.* Such grafts, whether autografts or homografts, were removed from anaesthetized rabbits by making a small incision with scissors at the vulval margins of the vagina and then stripping away the epithelium and some of the stroma from the underlying tissues by blunt dissection with the points of the scissors. A piece, about  $6 \times 4$  mm., was usually obtained from each of the four quadrants of the vaginal wall. They were placed in a Petri dish containing sterile normal saline until they were required. The period before grafting was never more than half an hour. Pl. 1, fig. 1, shows a section of the tissue prepared for grafting.



(b) *Grafts from the central vagina* (homografts only). The abdomen of the donor rabbit was opened, with aseptic precautions, by a subumbilical midline incision. Both uterine horns were cut through between clamps at their junction with the cervix and the bladder was removed after the urethra had been clamped. The symphysis pubis having been split open, the cervix and the vagina were dissected free and removed *en bloc*. The central region of the vagina (just distal to the opening of the urethra) was subsequently removed with scissors and opened out so that it formed a single epithelial surface. The epithelium and some underlying stroma were then dissected free from the muscle with scissors and trimmed for grafting.

(c) *Grafts from the upper vagina*. In preparing autografts the abdomen of an anaesthetized rabbit was opened, with full aseptic precautions; the uterine horns were ligated just above their junction with the cervix and the mass of cervix and the proximal 2-3 in. of the upper vagina were dissected free from the surrounding tissues. The vagina was then clamped distally and the block of tissue removed. The cut end of the vagina was occluded by a purse string suture and the abdominal wound was closed in layers with catgut sutures. The rabbit's chest was then prepared for the operation of grafting.

The cylindrical tube of upper vagina that had been removed was opened with scissors. The muscle coat usually contracted vigorously during this procedure and it was some minutes before a quiescent flat layer of tissue could be obtained. In early experiments, attempts were made to dissect away with scissors from the underlying muscle either a sheet of epithelium or circular grafts that had been marked out with a 9 mm. corkborer, but the preparations that were obtained were thick and not very satisfactory. It was later found that sheets of epithelium, with only very little connective tissue, could be simply prepared by gripping the surface of the cut piece of vagina at its free margin with fine-toothed forceps and pulling off a sheet of the epithelium, while maintaining tension on the underlying muscle layers with another pair of forceps. The thin membrane was then cut into smaller pieces for grafting, or was used as a single large sheet, up to  $4 \times 3.5$  cm. in size. Pl. 1, fig. 2, shows a section of the whole thickness of the upper vagina. The plane of separation is indicated by the arrow.

Homografts were obtained in a similar fashion, either from a rabbit that had just been killed or, if more grafts were to be needed at a later date, from an anaesthetized rabbit.

(d) In a few experiments sheets of epithelium from the cervix were also prepared in a similar fashion.

Some attempts were made to sterilize the epithelial lining of the vagina by instilling a solution containing 50,000 units penicillin and 500 units streptomycin into the vagina some time before the grafts were removed. This procedure did not appear to improve the chances of obtaining successful grafts and was abandoned.

#### *Grafting procedure*

A standard rectangular raw area (about  $6 \times 4$  cm.) was prepared on the side of the chest of the recipient in the usual way by stripping off the skin down to the level of the panniculus muscle. The individual pieces of tissue to be grafted were then placed

in position on the raw area, powdered with sterile sulphadiazine and covered with a fine tulle gras dressing. A layer of ointment, containing 30 mg. terramycin and 10,000 units polymyxin B per g., was smeared over the tulle gras and a dry gauze dressing, bandage and plaster of paris covering were then applied. Apart from the addition of the terramycin ointment, the technique was similar to that used for skin grafting (Billingham & Medawar, 1951). The area was first inspected 6 days after the operation and subsequently at intervals of 3 or more days. Biopsy specimens were cut out as required, fixed in Bouin's fluid and prepared for histological examination by standard methods.

#### *Treatment with hormones*

Male rabbits which were treated with oestrogen received 1 mg. oestradiol dipropionate daily by subcutaneous injection. Most of the female rabbits were given 0.5 or 1 mg. oestradiol dipropionate daily. Stilboestrol dipropionate was occasionally used.

### RESULTS

#### *Behaviour of the raw area*

The process of healing in untreated rabbits, as judged by the development of granulation tissue, the contraction of the margins of the wounds and the ingrowth of native skin epithelium was entirely normal. Healing was delayed in rabbits to whom oestrogen had been given.

#### *Behaviour of autografts*

*From the introitus.* The autografts were easily identified 6 days after the operation. They were usually pink, obviously vascularized and rather soft and flabby. They were often transparent and small blood vessels could be seen through the surface epithelium. A small fringe of outgrowing epithelium was sometimes already visible. Pl. 4, figs. 19-24, show the development of a single autograft in the centre of the raw area over a period of 32 days. The original graft tissue remains pink, soft and transparent. It becomes recessed below the level of the surrounding tissues, apparently because the granulation tissue underneath it fails to develop or thicken in the usual fashion. As a result a 'moat' forms which is covered by a thin layer of epithelium growing out from the original graft. At the margins of this outgrowth the granulation tissue thickens up normally. As time goes on the prepared graft bed contracts and skin epithelium grows in from the margins. Vaginal and skin epithelium come in contact, and gradually the ingrowing skin epithelium replaces the outgrowth from the vaginal graft until it has swept round the original graft and in about 20-25 days has surrounded it completely. By this time all traces of outgrowth of vaginal epithelium have vanished, but the original graft is usually able to maintain its individuality. Even as long as 148 days after the operation it has been readily identified, entirely surrounded by normal skin; it is usually covered with some mucus secretion. Sometimes, however, even the vaginal epithelium of the original graft becomes entirely replaced by skin epithelium which then grows over the characteristic stroma of the vaginal graft (Pl. 1, fig. 3). The stroma remains pink and intensely vascular but is covered by an opaque, keratinizing skin epithelium.

Histologically, the grafts always retain the special features of the vaginal stroma, its loose connective tissue, scattered muscle fibres, lymphatics and dilated numerous blood vessels, and become covered by a thickened, stratified epithelium (Pl. 1, figs. 4, 5). Where the epithelium is spreading out over the granulation tissue it becomes much thinner and consists usually of a single layer of very pale, high columnar epithelial cells covered by five or six layers of elongated, densely packed, stratified cells with very little cytoplasm and pyknotic spindle-shaped nuclei. The abrupt differentiation between these two layers is very apparent (Pl. 1, fig. 6). Sometimes, and especially when oestrogen has been given, proliferation is more active and the layer of outgrowth thickens and becomes more uniform in appearance.

The boundary zone where skin and vaginal epithelia come in contact is clearly shown in Pl. 2, figs. 7 and 8. The skin epithelium can readily be distinguished by the presence of pigment granules in the outer layers and by the layer of dead keratinized cells which is thrown off. The vaginal epithelium is not pigmented, does not keratinize and is much thinner. The nuclei of the outer layers are clearly pyknotic; those of the basal layer are pale, columnar and show far fewer mitoses than can be seen in the epidermal cells which stain more densely. Pl. 2, fig. 8, provides a clear impression of a tongue of epidermal cells that is infiltrating under and replacing the vaginal epithelium. It is noteworthy that the two epithelia do not intermingle at all. There is no sign in the form of a local reaction that any cells are being actively destroyed. It seems more likely that the vaginal epithelium is simply undermined and then cast off.

*From the upper vagina.* Sheets of epithelium soon become well attached and take on a cherry red colour as they become intensely vascularized by fine leashes of blood vessels which are clearly visible through the transparent vaginal epithelium (Pl. 4, figs. 25-27). They sometimes become corrugated and thickened and, as with autografts from the introitus, seem to inhibit the development of granulation tissue beneath them. There is usually no obvious outgrowth from the graft, but the margins may become thickened and ridged. Microscopically, these grafts (and also the few grafts of cervical epithelium that were used) retain their normal structure. Pl. 2, fig. 9, shows the characteristic epithelium and the large lymphatic vessels, just beneath the surface. It should be compared with Pl. 1, fig. 2, which provides a picture of the epithelium before grafting. A process of replacement, which is identical to that described above for squamous vaginal epithelium, takes place where the skin and the columnar epithelium come in contact (Pl. 2, fig. 10). In addition, the skin epithelium readily grows over the surface layer of columnar cells and the necks of the glands which have been formed by invaginations into the stroma. As a result of this process and perhaps of the contraction of the wound, complex glandular structures develop in the stroma which eventually become cystic when all contact with the exterior is prevented by the solid sheet of skin epithelium (Pl. 2, fig. 11; Pl. 3, fig. 13).

The junction between the two sorts of vaginal epithelia in preparations where columnar and stratified epithelia come to lie side by side is even more clear than that between skin and vaginal cells. There is no intermingling of the cells and no obvious attempt at replacement of one type by the other (Pl. 2, fig. 12).



A low power view of a section across the area pictured on Pl. 4, fig. 27, is shown in Pl. 3, fig. 13. The two types of vaginal grafts are seen in close contact with each other and with the skin at each outer margin.

Table 1. *Summary of experiments to study the survival of homografts and the behaviour of autografts*

Rabbit no. and sex	Body weight (g.)	Number of autografts from		Number of homografts from			Break- down time (days)
		Introitus	Other sites	Introitus	Central vagina	Upper vagina	
1, F.	2650	2	—	1	—	—	> 9
2, F.	4550	2	—	1	—	—	> 9
4, F.	3450	2	—	3	—	4	< 10
6, F.	3750	2	—	—	—	—	—
7, F.	2650	—	—	—	—	4+6*	< 12
8, F.	2150	—	—	—	—	4+5*	< 9
9, F.	2900	2	—	—	—	—	—
10, F.	2000	2	—	—	—	—	—
13, F.	2700	2	—	—	—	5	< 9
14, F.	2530	2	—	—	—	5	9
15, M.	3100	—	—	—	—	9	7
16, F.	3950	2	—	—	—	6	9
18, M.	2800	—	—	—	—	6	6
20, F.	3000	2	—	—	—	6	< 9
21, F.	2425	2	—	—	—	6	8
22, F.	2650	2	—	—	—	6	< 9
27, F.	2625	2	—	4	—	—	15
28, F.	2925	2	—	4	—	—	< 9
41, F.	3050	—	—	—	—	1	7
47, F.	3500	2	2†	2	—	—	10
49, F.	3000	2	4‡	—	—	—	—
50, F.	2790	2	—	2	—	3	8
63, F.	2800	1	—	2	2	2	< 9§
64, F.	2950	1	—	2	2	2	< 9§

\* Grafts from two donors, one spayed and one normal.

† From cervix.

‡ From cervix and epithelium from upper vagina.

§ No biopsies taken. Estimates from naked eye appearances only.

#### *The response of the vagina to the administration of oestrogen*

(a) *The normal vagina in situ.* The entire vagina becomes very vascular, oedematous and enlarged. Secretory activity in the columnar epithelial lining of the upper vagina is intense. The cells enlarge, their free margins are frayed and large quantities of mucus secretion are released into the lumen. The stratified epithelium of the lower vagina thickens. The outer layers become more squamous and more cells with pyknotic nuclei are shed. Obvious collections of leucocytes form just beneath the epithelium.

(b) *Vaginal autografts.* Apart from the experiments mentioned in Table 1, in which the autografts were used to control the technical adequacy of the operative procedure when studying the effect of oestrogen on the reaction of homografts, a further group of experiments was designed specially to study the behaviour of autografts, as indicated below. In each of these experiments pinch grafts were taken from the introitus and large sheets from the columnar epithelial lining of the upper vagina. Treatment with oestrogen was arranged so that rabbits

received the hormone (a) before and after, (b) before, (c) after grafting and (d) not at all.

Rabbit no.	Body weight (g.)	Oestrogen given		Period of observation (days)
		for 14 days before grafting	Oestrogen given after grafting	
65	3450	+	+	53
66	3525	+	—	17
67	2760	—	—	48
68	2650	—	+	21

The histological picture of the response of grafts to oestrogen was similar to that of normal vaginal tissue *in situ*. Whether the grafts had been removed from an animal previously treated with oestrogen or not made no obvious difference to the way in which they became attached to the graft bed. Outgrowth of epithelium from the introitus graft was increased in the treated rabbits. There was only a trace of outgrowth from the sheet of vaginal columnar epithelium but the surface of the graft became very corrugated and showed wart-like protuberances. Under the microscope, large adenomatous-like collections of glands could be seen where the columnar epithelium had proliferated (Pl. 3, fig. 14). The overgrowth by skin epithelium and subsequent cyst formation in glands whose mouths had been cut off by the epidermal ingrowth, mentioned above in describing the behaviour of the columnar epithelium in untreated rabbits, was not so evident; perhaps it was held in check by the extra growth activity of the epithelium.

#### *Behaviour of homografts*

A typical reaction rapidly develops against all types of homograft of the vagina no matter from what level they were obtained. Homografts become well attached to the graft bed and, to begin with, may proliferate and show a little outgrowth. But they soon become distinguishable from the autografts by the extent of the swelling, the deep purple coloration and the blotchiness of the vessels, which betoken vascular stasis and breakdown of the vessel walls. The distinction can usually be made at the first inspection 6 days after grafting and rapidly becomes more obvious as the homografts become blackened and hardened with necrotic yellowish margins that are undermined by ingrowing skin epithelium (Pl. 4, figs. 19–24). An unusual feature is found in grafts from the central vagina which, before grafting, contained a large number of blood sinuses. Vascular connexion between the vessels of the host and these sinuses seems to be established very rapidly and the sinuses become enormously distended with blood. The dark purple colour and the extreme swelling of the grafts is very evident when the area is first inspected 6 days after grafting. The slightest trauma during the removal of the dressings results in bleeding. Microscopically, such grafts are made up almost entirely of the widely dilated sinuses which at first contain stagnant blood. Later the integrity of the vessel walls is lost and the thrombi which are formed within the vessels gradually undergo a process of fibrous organization. Apart from this extraordinary development of blood sinuses the general histological picture of the homograft reaction to vaginal tissue is quite similar to that found with skin homografts. There is an early phase of epithelial activity, rapidly followed by a generalized infiltration with round cells, disorganiza-

tion and death of the graft's epithelium. The enlarged thrombosed blood vessels and the loose stromal elements can be identified for some time after the epithelium has been totally destroyed. Stages in the reaction are shown in Pl. 3, figs. 15-17.

Grafts from the upper vagina do not become swollen to the same extent (Pl. 4, fig. 19), but their thinness renders the blotchy vessels and stagnant circulation even more apparent. The usual picture of epithelial disintegration and lymphocytic infiltration is seen under the microscope (Pl. 3, fig. 18).

The survival time of the homografts has been estimated from the histological sections of biopsies, removed at intervals after the operation. The figures in Table 1 provide estimates of the day on which surviving homograft epithelium could no longer be identified. The estimates of survival time vary between 6 and 10 days, except in a few early experiments where the dose of homografted tissue was very small and survival a little longer. Such survival times are closely comparable with those obtained for skin homografts in rabbits. Biological tests of survival, by grafting back to the original donor pieces of vagina which have spent varying lengths of time in the foreign environment, have not yet been carried out satisfactorily.

*The effect of treatment with oestrogen on the response to homografts*

Seven paired experiments were carried out in which a single donor provided graft material for two recipients (five pairs of female and two pairs of male rabbits) one of which received oestrogen while the other remained untreated (Table 2). The life

Table 2. *Summary of experiments to study the effect of oestrogen on the behaviour of vaginal homografts*

Rabbit no. and sex	Body weight (g.)	Number of autografts from		Number of homografts from			Breakdown time (days)
		Introitus	Other sites	Introitus	Central vagina	Upper vagina	
30, F.	3050	2	—	3	—	3	<9
*31, F.	3100	2	—	3	—	3	6
33, F.	2900	2	—	3	3	3	10
*34, F.	3030	2	—	3	3	3	7
36, F.	3600	2	—	3	3	3	6
*37, F.	3500	2	—	3	3	3	7
39, M.	3450	—	—	3	3	3	7
*40, M.	3425	—	—	3	3	3	8
43, M.	3200	—	—	3	3	3	8
*44, M.	3200	—	—	3	3	3	8
45, F.	3550	2	—	1	3	3	7
*46, F.	3750	2	—	1	3	3	10
52, F.	3800	1	2†	2	—	2	9
*53, F.	3720	1	2†	2	—	2	<9

\* Animals receiving oestrogen.

† From cervix and from upper vagina.

of the homografts was not significantly prolonged by this treatment. The granulation tissue of the wound area developed more slowly than usual and at autopsy the adrenal glands were enlarged. It may be supposed, therefore, that treatment with oestrogen had stimulated production of adrenal cortical hormones, but the quantities produced were evidently unable themselves to affect the homograft reaction.



*The response to a second set of homografts (from the original donor)  
and the effect of oestrogen on this response*

It is known that a second set of skin grafts (from the original donor of a set of grafts transplanted previously) is destroyed more rapidly by the host than was the first set. Table 3 summarizes the experiments which were carried out to study the response of rabbits to second sets of vaginal homografts and to see whether previous

Table 3. *Summary of experiments to study the survival time of first and second sets of grafts of vaginal tissue*

(Rabbit 26 received grafts from the introitus only on both occasions. The other rabbits received grafts from the introitus only at the first operation but grafts from introitus, central and upper vagina at the second operation.)

Recipient rabbit no. and sex	Body weight (g.)	Interval between first and second operation (days)	Oestrogen given to donor before second operation	Oestrogen given to host after second operation	Breakdown time (days)	
					1st set	2nd set
26, F.	2550	42	—	—	7	< 6
51, F.	3370	45	—	+	8	6
56, F.	3430	39	+	—	8	< 6
58, F.	3670	46	+	+	8	< 6
60, F.	3350	38	—	—	9	< 6

treatment of the donor with oestrogen (to encourage proliferation of tissue that was to provide the grafts) or treatment of the host with oestrogen after grafting would have any effect on the survival of the homografts. The intensity of this accelerated reaction to the second series of grafts may begin to diminish if the interval between the two operations of grafting is more than about 3–4 months. In these experiments the interval between the two operations was 39–46 days. The results show that destruction of the second group of grafts is accelerated under all conditions of treatment with oestrogen as well as when no extra treatment is given.

#### DISCUSSION

The experiments have shown that autotransplants of vaginal tissue can be grafted without difficulty to a bed of granulation tissue prepared on the side of the chest. The normal histological structure of both stroma and epithelium is maintained except where the outgrowing epithelium becomes modified as it spreads in a thin layer over the graft bed of granulations. Not only tissue from the lower vagina, which is covered by a fairly tough squamous stratified epithelium, but also thin membranes, made up almost entirely of a single layer of columnar cells, can be grafted successfully and will survive the changed environmental conditions of the experimental procedure. Grafts of columnar epithelium do not, however, spread out over the graft bed in the same vigorous way as does stratified squamous epithelium. This lack of spreading activity contrasts with the very rapid resurfacing of the uterus by columnar epithelium which takes place under other circumstances, e.g. after parturition or menstruation. The grafts do, however, form thick glandular structures with the epithelium penetrating deeply into the stroma.

The only previous work in which vaginal tissue has been transplanted is that of Raynaud (1930), Martins (1932) and Arkelger & Huseby (1951). Raynaud grafted pieces from the central area of the spayed guinea-pig's vagina to the subcutaneous tissues of the back of the same animal, and showed that the grafts subsequently responded normally to the injection of oestrogen. Arkelger & Huseby (1951) have also used subcutaneous grafts of vagina in male mice of inbred strains as an indicator of the biological activity of injected hormones. Martins grafted the entire vaginal tube into the subcutaneous tissues of the back of male rats in such a way that a fistula was formed through which vaginal smears could be taken. He believed that the administration of oestrogen improved the 'taking' of the grafts and also showed that normal oestrous cycles could be obtained from the grafts if ovaries were also transplanted to the male recipient.

Martin's paper does not mention the genetic relationships of his host and donor pairs, and it must be presumed that the success of his experiments implies that the animals were derived from a closely inbred stock which could readily exchange grafts. Certainly the behaviour of homografts of vaginal tissue from unrelated rabbits, as reported here, is no different from the behaviour of homografts of skin. The type of reaction which destroys them is similar and the survival time is about the same. There is also some indication that the grafts survive longer when the total amount of tissue transplanted is small. An immunity is developed after the first set of grafts so that a second set is more rapidly destroyed than the first.

The columnar epithelium of the upper vagina seems just as capable of eliciting the homograft reaction as the tissues from the introitus or the central vagina which more closely resemble skin in that they are covered by a layer of squamous stratified cells. The results with this layer of epithelium indicate, as Billingham & Sparrow (1954) have already shown for grafts of pure epidermal cells, and Billingham & Boswell (1953) for corneal epithelium, that the antigenic stimulus comes mainly from the epithelium and not from the less nucleated connective tissue of the stroma. No attempts have been made to see whether the vagina and skin share any of the antigens which are responsible for the development of immunity.

Growth of autogenous vaginal epithelium is actively stimulated by oestrogen and should provide a satisfactory basis for testing the hypothesis that cellular proliferation can protect homografts from the consequences of the immune reaction. Despite the fact that the doses given were evidently sufficient to stimulate growth in autografts, homografts succumbed to the usual reaction just as rapidly and in the same general manner as did grafts in untreated rabbits. It is possible that the grafts had been destroyed before the oestrogenic stimulus could become effective but the results certainly do not encourage the view that the addition of growth stimulants is likely to help the survival of grafts. Rous (1946) has suggested that rapidly growing skin epithelium is more difficult to graft than less active skin. Whether the rabbit which provided tissue for grafting had earlier been spayed, had received no treatment or was being given oestrogen, appears to make no difference to the fate of the grafts or the ease of grafting.

The retardation of the formation of granulation tissue underneath grafts of vaginal tissue contrasts very sharply with the rapid growth of granulations beneath grafts of skin. The vaginal epithelium stains vigorously with the PAS reagent and may be

manufacturing considerable amounts of glycogen. The formation of acid breakdown products of this material might perhaps be inimical to the normal development of granulation tissue.

When ingrowing epithelium from the margins of a wound area comes in contact with the outgrowing epithelium from a skin autograft, the two fuse together to form a single sheet of epidermal cells. Later the entire sheet disappears as further contraction around the graft takes place. What happens to the epithelium during the process is still quite unknown (Billingham & Reynolds, 1952). Outgrowing vaginal epithelium, on the other hand, is unable to maintain a balance with the ingrowing skin epithelium and is replaced by undermining epidermal cells. The process seems to be identical with that which occurs when the outgrowth of corneal epithelium from a transplant to the side of the chest comes in contact with and is replaced by the ingrowing skin epithelium (Billingham & Medawar, 1950). As far as is known, the original epithelium covering a skin graft remains intact. Similarly, a vaginal graft is usually able to maintain the normal epithelial covering to the full-thickness graft, but sometimes even this proves impossible and the vaginal epithelium covering the graft itself is replaced.

Problems of the maintenance of a balance between two types of epithelium arise in many sites where they become contiguous. Smith (1913) studied such relations in experiments in which epithelia from the gall bladder, urinary bladder and uterus were grafted from one organ to the other. He also refers to earlier work of a similar nature. Smith found that the extent of replacement varied with the different types of epithelium and with the extent to which the normal environment of the epithelium was upset by secretions which were destructive to it in its new site. Epithelium from the uterus which was grafted into the gall bladder was unable to tolerate the continuous presence of bile. On the other hand, it was not affected if urine from a ureter implanted into the uterus flowed over it. An undisturbed epithelium usually overgrew a grafted epithelium. When Smith formed composite cysts of two epithelia by grafting both of them together, he found that they maintained a satisfactory balance without one replacing the other. Similar cysts containing both columnar and stratified epithelium have been formed where the two types of vaginal epithelium have been grafted close together on the chest wall. It is worth mentioning here that Billingham & Medawar (1950) also found that, when two outgrowing epithelia come into contact with each other, the final result depended on which one of the two was growing in its normal environment. Thus, corneal epithelium transplanted to the side of the chest was replaced by local ingrowing skin epithelium but, when growing in its normal environment, was able to replace the epithelium that grew out from a skin graft placed on the cornea.

One of the most important epithelial boundaries is to be found in the neighbourhood of the cervical os in women, where the line of demarcation between the vaginal and cervical epithelia moves up and down in relation to changes in the hormonal environment and where the invasive characteristics of active vaginal squamous epithelium, which replaces the cervical epithelium, may be concerned in the genesis of cervical carcinoma. Similarly, after a tracheotomy, there may be an ingrowth of squamous epithelium which replaces the columnar epithelium of the trachea. The erosion and replacement of uterine epithelium by the trophoblast cells during the



early stages of implantation may also represent a somewhat analogous situation. The observation of grafts that have been transplanted to areas of granulation tissue on the side of the chest wall may well provide a useful method for the study of the problem of metaplastic transformation and of the interaction of different types of epithelia under various experimental conditions.

#### SUMMARY

1. Autografts and homografts of the vagina of rabbits have been transplanted to prepared areas of granulation tissue on the side of the chest. Both male and female rabbits have served as recipients for the homografts.

2. The behaviour of autografts is described. Grafts from the introitus (derived from the urogenital sinus) which are covered by a squamous stratified epithelium, behave very similarly to grafts of skin transplanted to the same site. An expanding ring of epithelium grows out from the margins of the graft and is replaced by epidermal cells when the outgrowth comes in contact with ingrowing skin epithelium. All traces of spread epithelium are removed but the stroma of the original graft usually persists with a covering of its own epithelium.

Thin grafts from the upper vagina (derived from Müllerian tissues), consisting of a single layer of mucus secreting columnar epithelial cells and a few stromal elements, can also be transplanted satisfactorily. Such grafts persist and develop glandular ingrowths into the underlying stroma. There is little if any epithelial outgrowth.

3. Both kinds of epithelium respond to the administration of oestrogen by proliferation and (in the case of columnar epithelium) by mucus secretion. The responses of grafted tissues are similar to those of the epithelia *in situ*.

4. Administration of oestrogen before grafting does not affect the success of a subsequent grafting operation.

5. Homografts of the vaginal tissues, whether derived from the proximal (Müllerian) or distal (urogenital sinus) parts of the organ, call forth and succumb to an immunity reaction comparable in histological appearances and time relations to that elicited by skin.

6. A second set of vaginal homografts from the same donor breaks down more rapidly than the first set.

7. It has been suggested that rapid growth, induced by pituitary trophic hormones, may enable homografts of endocrine glands to offset the consequences of the homograft reaction. Treatment of homografts of the vagina with oestrogen to encourage their growth did not, however, prolong their survival.

#### REFERENCES

- ARKELGER, S. W. & HUSEBY, R. A. (1951). Estrogen-androgen antagonism: histology of mammary glands and vaginal grafts of male mice receiving estrogens. *Proc. Soc. exp. Biol., N.Y.*, **76**, 811-817.
- BAXTER, J. S. (1933). Development of the vagina in the rabbit. *J. Anat., Lond.*, **67**, 555-562.
- BILLINGHAM, R. E. & BOSWELL, T. (1953). Studies on the problem of corneal homografts. *Proc. Roy. Soc. B*, **141**, 302-406.
- BILLINGHAM, R. E. & MEDAWAR, P. B. (1950). A note on the sensitivity of the corneal epithelium. *J. Anat., Lond.*, **84**, 50-56.

- BILLINGHAM, R. E. & MEDAWAR, P. B. (1951). The technique of free skin grafting in mammals. *J. exp. Biol.* **28**, 385-402.
- BILLINGHAM, R. E. & REYNOLDS, J. (1952). Transplantation studies on sheets of pure epidermal epithelium and on epidermal cell suspensions. *Brit. J. Plast. Surg.* **5**, 25-36.
- BILLINGHAM, R. E. & SPARROW, E. M. (1954). Studies on the nature of immunity to homologous grafted skin with special reference to the use of pure epidermal grafts. *J. exp. Biol.* **31**, 16-39.
- CARLETON, H. M. (1931). Studies on epithelial phagocytosis. *Proc. Roy. Soc. B*, **108**, 1-10.
- CARR, E. B. (1953). The development of the rabbit vagina. *J. Anat., Lond.*, **87**, 423-431.
- HARRIS, M. & EAKIN, R. M. (1949). Survival of transplanted ovaries in rats. *J. exp. Zool.* **112**, 131-164.
- JOST, A. (1943). Sur le vagin de la lapine. *C.R. Soc. Biol., Paris*, **137**, 329-393.
- KROHN, P. L. (1955). Homotransplantation of ovarian tissue. *Ann. N.Y. Acad. Sci.* **59**, 443-447.
- MARTINS, T. (1932). Greffes de vagin sur des rats mâles, et observation des cycles au moyen des frottis. *C.R. Soc. Biol., Paris*, **109**, 134-135.
- MEDAWAR, P. B. (1944). The behaviour and fate of skin autografts and skin homografts in rabbits. *J. Anat., Lond.*, **78**, 176-199.
- MEDAWAR, P. B. (1945). A second study of the behaviour and fate of skin homografts in rabbits. *J. Anat., Lond.*, **79**, 157-176.
- RAYNAUD, R. (1930). Les autogreffes de muqueuse vaginale chez le Cobaye, leur sensibilité à la folliculine. *C.R. Soc. Biol., Paris*, **104**, 284-285.
- ROUS, P. (1946). The activation of skin grafts. *J. exp. Med.* **83**, 383-399.
- SMITH, G. M. (1913). Morphological changes in tissue with changes in environment. Replacement of surface epithelium of grafted tissues by adjacent epithelium. *J. Med. Res.* **28**, 423-439.

## EXPLANATION OF PLATES

## PLATE 1

- Fig. 1. Tissues from the lower vagina as prepared for grafting.  $\times 40$ .
- Fig. 2. Section through the upper vagina showing the high columnar epithelium, the subjacent large lymphatic channels and the thin layers of loose connective tissue between the epithelium and the thick muscular coat. The epithelium was stripped off through this layer during preparation of the graft (at the level of the arrow).  $\times 40$ .
- Fig. 3. Replacement of original vaginal epithelium by ingrowing skin epithelium which has formed a complete cover over the stroma of the graft.  $\times 59$ .
- Fig. 4. Autograft 9 days after operation. The graft is covered by an actively proliferating epithelium; the stroma is oedematous and the blood vessels are dilated. The rabbit was receiving oestrogen.  $\times 40$ .
- Fig. 5. Proliferating epithelium from an autograft 15 days after transplantation.  $\times 120$ .
- Fig. 6. Spread epithelium from an autograft. There is a narrow layer of pale columnar cell nuclei which is covered by several layers of stratified tightly packed pyknotic nuclei.  $\times 390$ .

## PLATE 2

- Fig. 7. Junction of ingrowing skin epithelium (right) and outgrowing vaginal epithelium (left).  $\times 120$ .
- Fig. 8. Junction of ingrowing skin epithelium (right) and outgrowing vaginal epithelium (left).  $\times 95$ .
- Fig. 9. Autograft of columnar epithelium 12 days after grafting.  $\times 40$ .
- Fig. 10. Junction of normal skin epithelium (right) with high columnar epithelium of graft from upper vagina.  $\times 59$ .
- Fig. 11. Ingrowing skin epithelium replacing the surface epithelium of a graft and covering over glands which had formed in the stroma. A cystic gland can be seen on the extreme right of the section.  $\times 59$ .
- Fig. 12. Junction between squamous and columnar-celled epithelia derived from two vaginal grafts placed close to each other.  $\times 95$ .

## PLATE 3

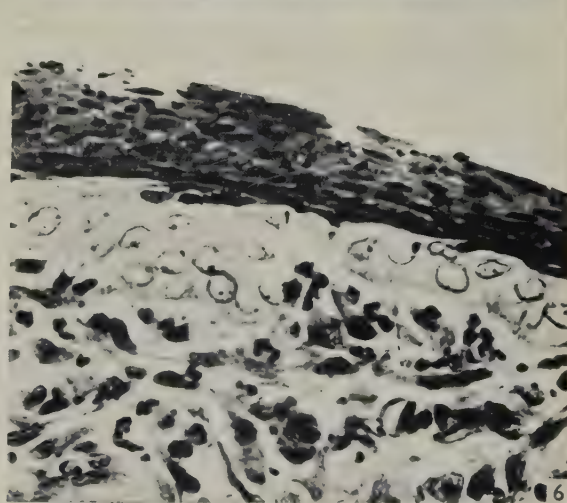
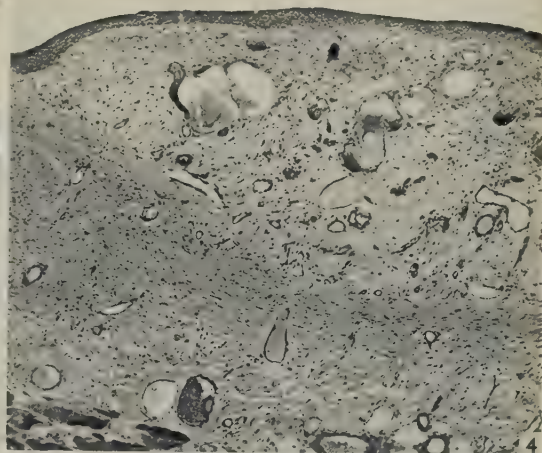
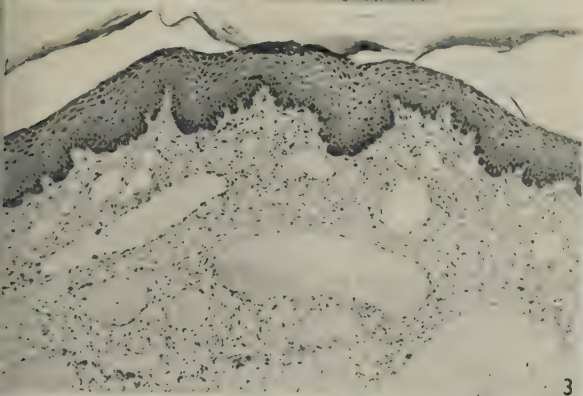
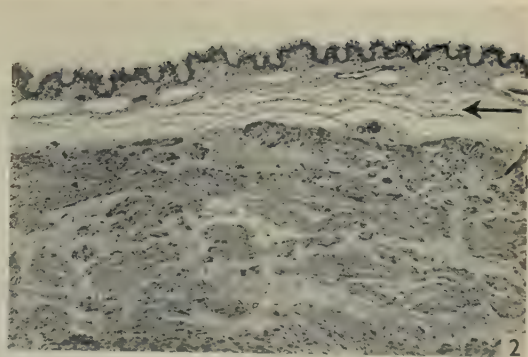
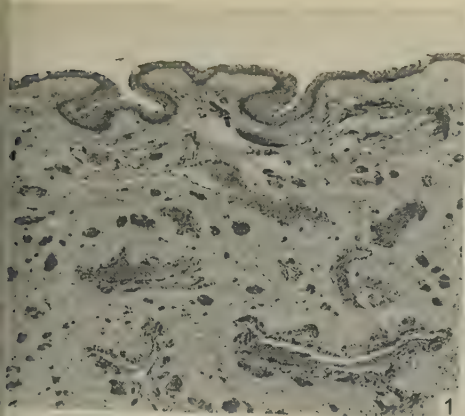
- Fig. 13. A low-power view of the area of the chest shown in Pl. 4, fig. 27. The following structures can be seen from right to left: normal skin; a graft from the upper vagina with adenomatous growths; a graft from the lower vagina with large blood vessels and a thick stratified epithelium; cystic dilated glands; normal skin.  $\times 5.5$ .
- Fig. 14. Complex glandular arrangement in a graft of upper vaginal tissue.  $\times 59$ .
- Fig. 15. Homograft from the lower vagina 6 days after grafting. The epithelium of the graft is still alive. The stroma is oedematous and the blood vessels are dilated. Round cells are beginning to accumulate around the margins of the vessels and to infiltrate through the stroma. The rabbit was receiving oestrogen.  $\times 40$ .
- Fig. 16. Homograft from the lower vagina 15 days after operation. The epithelium comprises a thin layer which is just disintegrating. There is widespread round cell infiltration in an oedematous stroma. The photograph shows the maximum survival of homograft tissue which has been obtained.  $\times 120$ .
- Fig. 17. Homograft from the central vagina 12 days after operation. From the same animal as fig. 15. The graft has been completely destroyed and is made up of large thrombosed blood sinuses in a stroma that is infiltrated with degenerating round cells.  $\times 40$ .
- Fig. 18. Homograft from the upper vagina 6 days after grafting. The columnar epithelium of the graft can still be recognized and it is still PAS positive. The stroma is not very swollen but is extremely vascular and moderately infiltrated with round cells. The graft is in the midst of a specific reaction. The rabbit was receiving oestrogen.

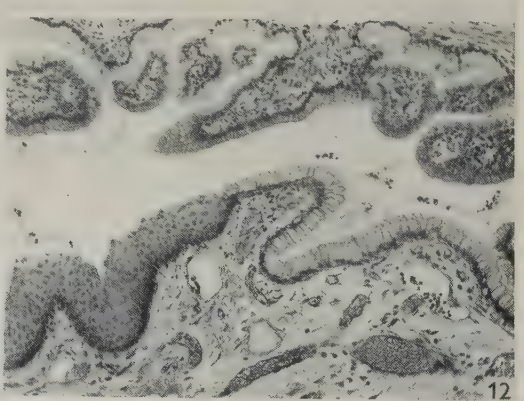
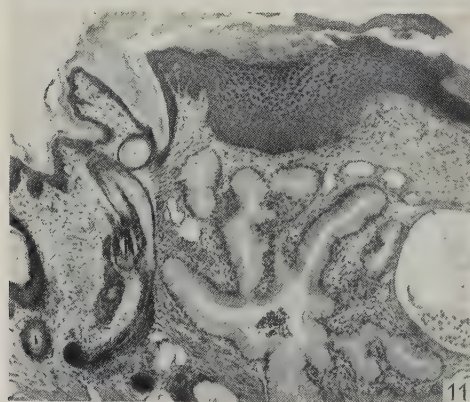
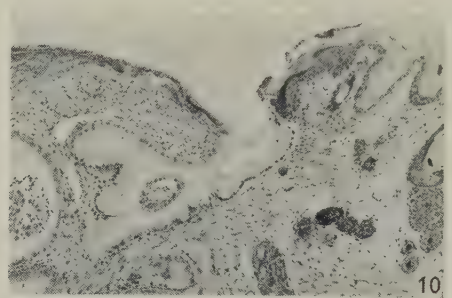
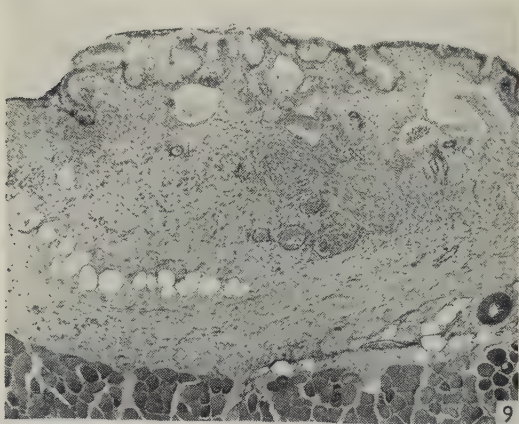
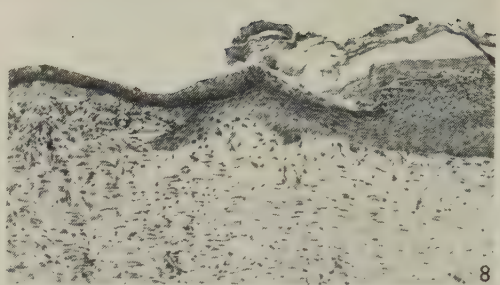
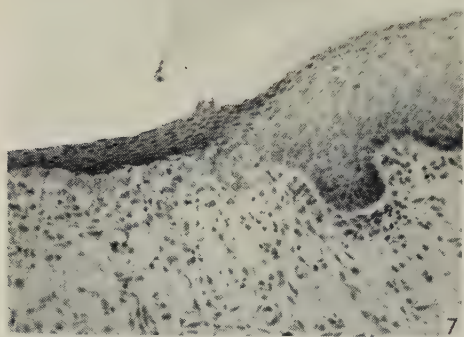
## PLATE 4

- Figs. 19-24. The area on the side of the chest photographed 9, 18, 22, 25, 29 and 32 days after grafting. A single autograft from the introitus develops in the centre, four homografts from the central vagina become necrotic and undermined at each corner and two sheets of homograft epithelium from the upper vagina, between the two rows of homografts, become blotchy (fig. 19) and have disappeared by 18 days (Fig. 20).
- Fig. 25. The chest 7 days after grafting showing a small vascularized autograft from the lower vagina (above) and a vascularized sheet of epithelium from the upper vagina (below). Granulation tissue in the bed of the wound has failed to develop in this specimen because of the onset of severe gastro-enteritis, and there has been no outgrowth.
- Figs. 26, 27. Development of a large sheet of upper vaginal epithelium (below) and a small pinch graft from the introitus (above) photographed 10 and 35 days after operation. Note the extreme vascularity of the graft in fig. 26 and the overgrowth of the vaginal epithelium by ingrowing skin in fig. 27.

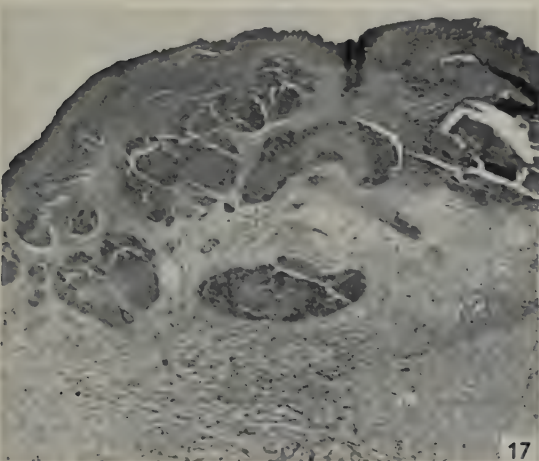
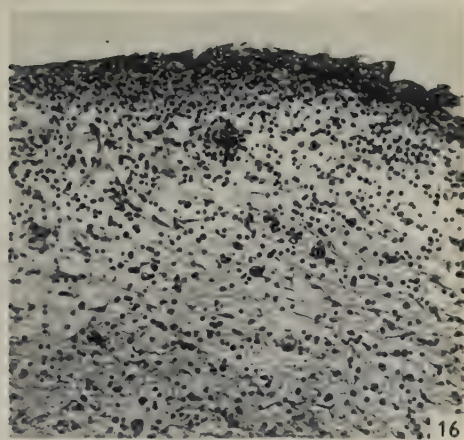
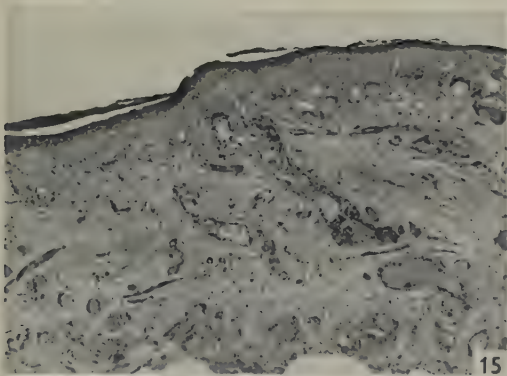
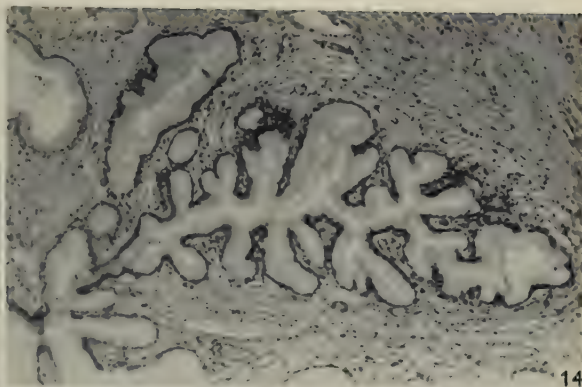
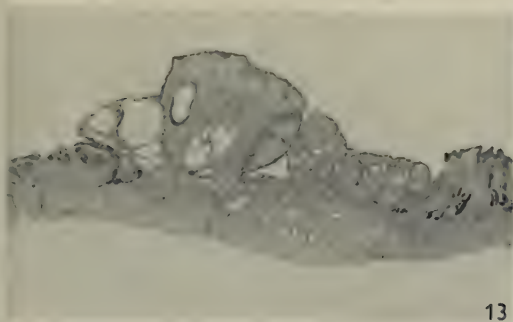
(Kodachrome transparencies taken with a Leica camera. Reduction: figs. 19-21 and 25, 1:3; figs. 22-24, 26 and 27, 1:1.5.

















19



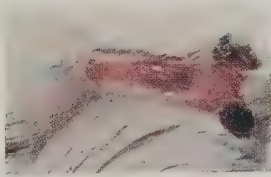
20



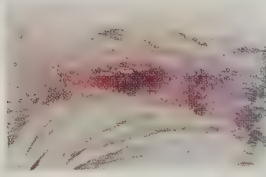
21



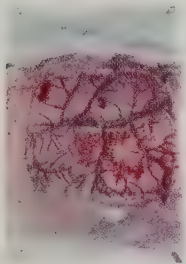
22



23



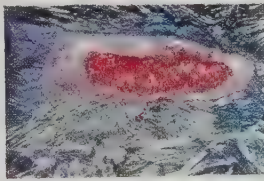
24



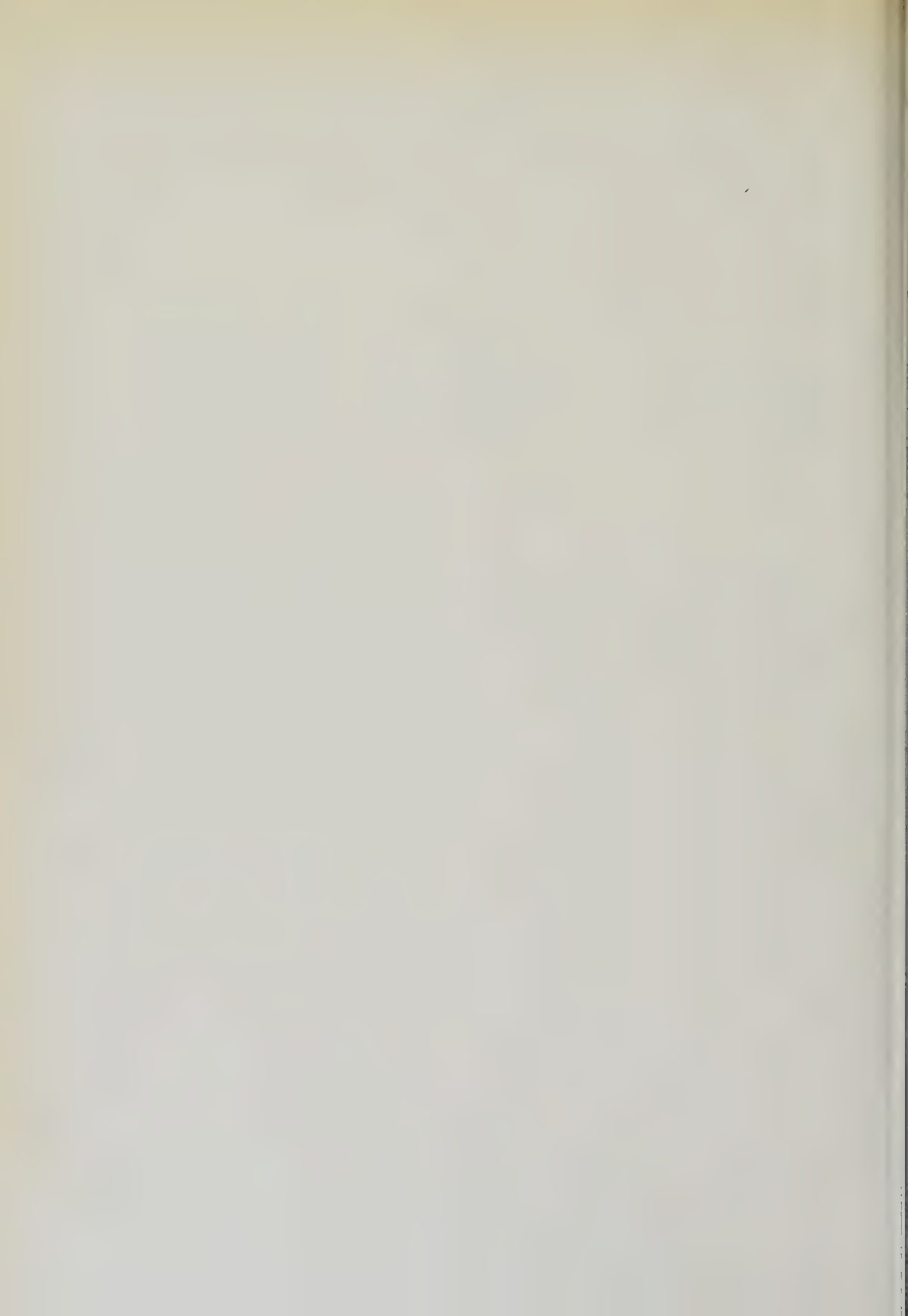
25



26



27





# CELLULAR CHANGES IN LYMPH NODES AND SPLEEN FOLLOWING SKIN HOMOGRAFTING IN THE RABBIT

By R. J. SCOTHORNE AND I. A. MCGREGOR

*Department of Anatomy, University of Glasgow and the Unit of Plastic Surgery,  
Royal Infirmary, Glasgow*

## INTRODUCTION

Skin transplanted between genetically dissimilar members of the same species survives for a time, but is sooner or later destroyed. This destruction is now known to be due to an actively acquired immune response on the part of the host (for recent review see Medawar, 1954), and much of the modern work on skin homografting has been concerned with testing the validity of the comparison between the immune state responsible for homograft destruction and that appearing in response to bacterial and other antigens.

Purely serological methods have so far failed to demonstrate antibodies against tissue homografts, but Billingham & Sparrow (1954) have shown by other methods the presence of a 'protective iso-antibody' in the serum of rabbits which have reacted against skin homografts. Since the pioneer work of McMaster & Hudack (1935), it has become increasingly evident that the lymphatic tissue is the main site of formation of the antibodies of classical immunology, and the work of Mitchison (1954) and of Billingham, Brent & Medawar (1953), on the passive transfer of transplantation immunity, has shown that the lymph nodes are also involved in transplantation immunity. In view of these facts, one might expect to find changes in the lymphatic tissue following skin homografting, and this investigation was designed to determine the character and distribution of these changes.

Gallone, Radici & Riquier (1952) reported briefly an increase in pyronin-staining elements in the regional node following skin homografting, and concluded that the response is similar to that following injection of known antigens. They did not analyse in any detail the cellular character of the response, nor did they report upon the spleen, or lymph nodes other than that immediately regional to the graft.

## MATERIAL AND METHODS

Rabbits of both sexes were used, the majority being young animals weighing between 1.5 and 2.5 kg. In the homografting experiments, skin was exchanged between unrelated animals to ensure a maximal immune response.

### (1) *Skin homografts*

A single full thickness graft (30 × 15 mm.) was cut from the dorsum of the ear and exchanged with a similar piece of skin from another rabbit. Each graft was secured by silk sutures. No dressings were used.

In all, sixteen rabbits received skin homografts. In one of these, initial 'take' of the graft was unsatisfactory and it was therefore discarded.

*(2) Skin autografts*

In a second group of animals a full thickness graft of ear skin ( $30 \times 15$  mm.) was cut from one ear, immediately replaced in the bed from which it had been cut, and sutured in position.

In all, eight rabbits received skin autografts. In four of these animals the graft was excised some 3–4 weeks later and again replaced and resutured. This was done to enhance any possible non-specific effect of the operative procedure on the lymphatic tissues.

*(3) Lymphatic injections*

To find whether the lymph from the operation site drained only to the ipsilateral nodes or to those of both sides, about 0.1 ml. of a 2% aqueous solution of pontamine sky blue was injected subcutaneously in one ear only of each of seven unoperated control animals. There was seen almost immediately a blue coloration of the lymphatics running from the injection site to the regional nodes; in every case blue staining was confined to the nodes of the injected side. The functional lymphatic drainage of the skin and subcutaneous tissue of the dorsum of the ear is therefore entirely ipsilateral.

These injected animals also provided material for a study of the position and form of the regional lymph nodes of the ear. The first regional node (node I) was very constant in form, size and position. It was always macroscopically single, and was found between the angle of the mandible and the base of the ear cartilage, partially embedded in fat and related to the parotid salivary gland.

The second node in the chain receiving lymph from the dorsum of the ear (node II) was found in the angle of union of the anterior and posterior facial veins. This node was less constant in size and form than node I. It was sometimes double and even when single was frequently only partially stained by injected dye.

No intermediate nodes were found between nodes I and II.

*(4) Controls*

A final group of six rabbits provided a normal control for weight and cellular content of lymph nodes and spleen.

Grafted animals were killed at intervals (Table 1). Nodes I and II of each side, the spleen and the graft itself were removed, fixed in 4% neutral formaldehyde, embedded in paraffin and sectioned at  $8\mu$ . The sections were stained with methyl-green-pyronin. Node I of each side, and the spleen, were weighed prior to fixation. Node II was not weighed because of its variability of form.

The means and standard deviations of the weights of nodes and spleen in the homografted animals were calculated on the basis of those animals marked †, i.e. those carrying homografts for 4, 5 and 6 days and in which the grafts were still healthy. This was done to make the figures directly comparable with those obtained in autografted animals killed at 4, 5 and 6 days, and to avoid as a possible source of error any non-specific changes in weight of lymph nodes or spleen which might occur in animals bearing dead graft tissue.

Table 1. *Weight of Regional Lymph Nodes and of Spleen*A. *Homografted animals*

Serial no.	Days after grafting	Condition of graft	Day of on-set of graft destruction	Weight of node 1 (operated side, in mgm.)	Weight of node 1 (control side, in mgm.)	Weight of spleen in g.
R 359	2	Healthy	—	205	120	1.97
L 309	2	Healthy	—	110	60	1.30
L 296†	4	Healthy	—	320	200	1.67
L 298†	4	Healthy	—	230	160	1.45
R 319†	4	Healthy	—	370	100	1.75
R 353†	4	Healthy	—	330	200	1.55
L 286†	5	Healthy	—	300	110	2.17
R 354	5	Early breakdown	5	750	190	1.67
R 355†	6	Healthy	—	350	180	1.83
R 350	6	Early breakdown	6	410	170	1.34
R 346	6	Early breakdown	6	285	120	1.75
R 358	7	Early breakdown	7	375	105	1.56
L 268	7	Advanced breakdown	6	Not weighed	Not weighed	Not weighed
L 264	10	Advanced breakdown	6	Not weighed	Not weighed	Not weighed
L 262	10	Advanced breakdown	8	Not weighed	Not weighed	Not weighed
Mean and s.d. (calculated for animals marked †)				317 ± 48.9	158 ± 44	1.74 ± 0.28

B. *Autografted animals*

Serial no.	Days after grafting	Condition of graft	Weight of node 1 (operated side, in mgm.)	Weight of node 1 (control side, in mgm.)	Weight of spleen in g.
R 310	4 (second set)	Healthy	270	180	1.60
R 312	4 (second set)	Healthy	230	170	1.40
R 321	4 (second set)	Healthy	200	120	1.30
R 13	4 (second set)	Healthy	150	100	1.05
L 292	5	Healthy	165	140	1.21
L 305	5	Healthy	120	70	1.27
R 360	6	Healthy	120	95	1.03
R 337	6	Healthy	150	80	1.67
Mean and s.d.			176 ± 55.1	119 ± 40.2	1.32 ± 0.23

C. *Unoperated controls*

Serial no.	Weight of node 1 (right, in mgm.)	Weight of node 1 (left, in mgm.)	Weight of spleen in g.
L 289	130	140	1.37
R 349	100	110	1.09
R 356	140	170	1.85
L 367	150	130	1.20
R 323	Not weighed	Not weighed	1.35
R 309	Not weighed	Not weighed	1.65
Mean and s.d.	130 ± 21.6	138 ± 25.0	1.42 ± 0.3



## HISTOLOGICAL FINDINGS

A. *Nodes from control animals*

The general topography of node I is shown in Pl. 1, fig. 1. The cortex consists of dense lymphatic tissue, incompletely divided into the so-called tertiary nodules of Ehrlich (1946). Small primary nodules are scattered through the cortex, mostly in a subcapsular position. In the cortex the only cells whose cytoplasm is stained with pyronin are the medium and large lymphocytes of the germinal centres, and a few medium-sized lymphocytes scattered through the tertiary nodules. By contrast, the cells of the slender medullary cords are noticeably pyroninophilic, even under low-power examination. They are mostly mature plasma cells, characterized by an eccentric nucleus, large, pale juxta-nuclear vacuole and intensely pyroninophilic cytoplasm. Node II is essentially similar to node I microscopically.

B. *Nodes from homografted animals*(1) *Two days after grafting*

At this time the graft is histologically normal, and the epidermis has not yet entered the stage of cell proliferation and hypertrophy characteristic of both autografts and homografts.

There is enlargement of both tertiary cortical nodules and medullary cords in node I of the operated side, but the primary nodules do not differ from those of unoperated controls. In the enlarged medullary cords pyroninophilic cells are more numerous. Most of them are mature plasma cells, but some are larger, with large pale nucleus, prominent nucleolus and abundant pyronin-stained cytoplasm. In the cortex there is no significant change in content of pyronin-staining cells. In the medullary sinuses there are large numbers of free macrophages packed with intact erythrocytes. These are a constant feature of nodes I and II on the operated side, in both homografted and autografted animals, at all stages. Since they are unusual in normal controls and in the nodes from the unoperated side of grafted animals, their presence in the nodes of the operated side confirms the fact that the lymph drainage of the operation site is ipsilateral.

In node II of the operated side there is slight enlargement of the tertiary cortical nodules and medullary cords. The number of mature plasma cells is somewhat increased in the medullary cords. Nodes I and II of the unoperated side are essentially normal.

(2) *Four days after grafting*

In all four animals killed at this stage the graft was healthy histologically. The epidermis was considerably thickened, as a result of cellular hypertrophy and hyperplasia.

Pl. 1, fig. 2, shows a low power view of node I from the operated side. The enlargement of the node involves mainly the tertiary cortical nodules. There is, moreover, a striking change in the cellular composition of the node. The cortex is packed with large lymphoid cells. These are round or ovoid in outline, about 15–17  $\mu$  in greatest diameter, and have large pale nuclei, one or more prominent nucleoli, and strongly pyroninophilic, rather vacuolated, cytoplasm (Pl. 2, fig. 6). Their distribution is

fairly uniform and they appear to have developed *in situ* from cells of the tertiary nodules, and not through hypertrophy and extension of the germinal centres, which remain normal in size and subcapsular in position.

The medullary cords are enlarged, but less so than the cortical nodules, and contain an increased number of mature plasma cells and moderate numbers of large lymphoid cells similar to those in the cortex. In the medullary sinuses there are many small lymphocytes and a few large lymphoid cells.

These cellular changes are virtually confined to node I of the operated side. In node II of the operated side and nodes I (Pl. 1, fig. 3) and II of the unoperated side there is a minimal enlargement of cortical nodules and medullary cords, and only a slight increase in the numbers of mature plasma cells in the medulla. In cortex and medulla there are a few scattered large lymphoid cells.

### (3) *Nodes removed at onset of graft destruction*

Four animals were sacrificed when graft breakdown was first apparent on naked-eye inspection. In each case microscopic study confirmed the fact that graft breakdown was of recent onset.

Pl. 2, fig. 7, shows part of a tertiary cortical nodule in node I, operated side, of an animal (R 350) in which graft destruction had just begun. It illustrates the abundance of large lymphoid cells, similar to those seen at 4 days. In a corresponding area of node I on the unoperated side, these cells are absent (Pl. 2, fig. 8).

### (4) *Nodes removed when graft breakdown is advanced*

Animals L 264 and L 262 were sacrificed on the 10th day after grafting, 4 and 2 days respectively after the time of onset of graft breakdown, as determined by naked-eye inspection. In both cases microscopic study of the graft confirmed the fact that destruction was advanced.

Pl. 1, fig. 5, is a low-power view of node I of the operated side from animal L 264, 4 days after the onset of graft destruction. The cortical nodules and medullary cords are still enlarged, but the number of large lymphoid cells is much reduced in the tertiary nodules (Pl. 2, fig. 9), where the small lymphocyte is, as in normal nodes, the predominant cell. In the medullary cords the numbers of both mature plasma cells and large lymphoid cells are reduced, and some areas of the medulla are markedly depopulated of cells. The medullary sinuses contain fewer cells than in the 4-day specimen, those present being mainly small lymphocytes.

In node I of the unoperated side the histological appearances are essentially similar to those described in the comparable node at 4 days. Node II was not examined at this stage.

### C. *Nodes from autografted animals*

Nodes from autografted animals were studied at 4, 5 and 6 days after grafting, i.e. at the time of maximal large lymphoid cell response in homografted animals. In each case the graft was healthy, both grossly and microscopically.

Node I of the operated side (Pl. 1, fig. 4) was in most cases larger than normal, but the enlargement was much less than that seen in homografted animals. The tertiary nodules were slightly enlarged. In some cases they showed more large lymphoid cells than in normal controls, but very few indeed by comparison with the corre-

sponding nodes from homografted animals. In many areas of the cortex, large lymphoid cells were entirely absent (Pl. 2, fig. 10).

On both operated and control sides the medullary cords contained more mature plasma cells than normal, but fewer than in homografted animals.

#### *D. The spleen*

Thorough histological examination failed to show any significant differences in the spleens of autografted, homografted and normal control animals.

#### DISCUSSION

From the results presented in Table 1, it is evident that the maximum response of the lymphatic tissue to a skin homograft occurs in the node regional to the graft site. Since the weight increase in this node is greater in homografted than in autografted animals it is not merely a non-specific effect of the operation, but a specific response to some factor reaching it from the homograft. (The term 'factor' is used in the singular, here and elsewhere in this discussion, purely for convenience. It is not intended to imply that only a single factor or antigen is involved. There is, indeed, clear-cut evidence to the contrary (v. Medawar, 1945)). Moreover, the greater increase in weight of the node in homografted animals cannot be attributed to 'toxic material' appearing in the graft at the time of graft breakdown, since it is already evident before breakdown begins.

The figures in Table 1 also indicate that the 'homograft factor', whatever it may be, reaches the regional lymph node principally by way of the lymphatics. If it were blood-borne one would expect an equivalent weight increase in the contralateral node and a greater increase in splenic weight than does in fact occur.

These conclusions are fully supported by the histological findings in lymph nodes and spleen. In the first regional node draining a homograft site there develops a characteristic 'large lymphoid cell' response in the tertiary nodules of the cortex and, to a lesser extent, in the medullary cords. This response is not evident at 2 days, is fully developed at 4 days, and remains so in animals in which graft destruction is in its early stages. When graft destruction has been in progress for 2 or more days the cellular response is markedly reduced. The response is very largely confined, at all stages, to the first regional node of the operated side.

The 'large lymphoid cell' is rare in normal control nodes, and is found in relatively very small numbers in autografted animals. Thus, while the 'large lymphoid cell' response is not, in the strict sense of the term, specific to the homografted animal, from a quantitative point of view it is undoubtedly a homograft response.

The 'large lymphoid cell' response was not seen in the spleen; indeed neither homografting nor autografting appeared to produce any histological change in the spleen. This negative finding confirms the impression, gained from study of the weight changes, that the spleen is little affected by orthotopic skin homografts.

The findings of this investigation are all consistent with the view that some factor, presumably antigenic in nature and specific to the homograft, leaves the homograft and travels by way of the lymphatics to the first ipsilateral lymph node, and that little of this 'homograft factor' appears to reach the contralateral nodes or the spleen.



The significance of these findings is clearer in the light of some recent experimental studies. Mitchison (1954) has shown that, in mice, immunity to certain tumours may be transferred passively by the injection into non-immune animals of a suspension of minced lymph nodes from immune animals. Moreover, passive immunity was transferred by suspensions of cells from the regional nodes of the operated side only, and neither by cells from nodes of the opposite side nor by splenic tissue. These findings have since been shown by Billingham *et al.* (1953, 1954) to be directly applicable to skin homograft immunity, with the sole difference that the spleen was found to be weakly effective in the passive transfer of homograft immunity. While, on a dosage basis, the power of the spleen to transfer immunity is much weaker than that of the regional nodes, the fact that it is effective at all implies, of course, that some 'homograft factor' does reach it. Furthermore, it should be remembered that the immunity to homologous skin is systemic (Medawar, 1945), and that a second graft of skin from the same donor will be destroyed more rapidly than the first, regardless of the site to which it is transplanted. It seems likely, therefore, that the 'homograft factor' does reach lymphatic tissues other than the first regional node in quantities too small to produce histologically demonstrable change, but large enough to 'sensitize' them, so that a second exposure to skin from the same donor elicits a heightened response, comparable with the 'anamnesic response' of classical immunology. This supposition is supported by the finding of Billingham *et al.* (1954) that a second-stage graft transplanted to the other side of the body breaks down at its usual accelerated rate even if the regional nodes are excised after the breakdown of the first-stage graft.

The role of the spleen in homograft immunity has also been investigated by Krohn & Zuckerman (1954), who found that splenectomy did not prolong the survival of skin homografts in rabbits.

These findings, taken in conjunction with our own, suggest that the 'large lymphoid cell' response may play an active part in homograft destruction. Antibodies are certainly involved in homograft destruction, and the 'large lymphoid cell' bears at least a family resemblance to the cells implicated by various authors in the production of antibodies against known antigens. In general, three cell types have been suggested as antibody producers: the macrophage, the lymphocyte, and the plasma cell or its progenitors (v. McMaster, 1953). The evidence in favour of the macrophage is largely circumstantial (v. Sabin, 1939), and the work of Ehrich, Harris & Mertens (1946) argues strongly against it. The lymphocyte has found many supporters: Harris & Harris (1948, 1949), Harris, Grimm, Mertens & Ehrich (1945) and Dougherty and White (1947), among others, and at present attention is focused upon the plasma cell or its progenitors (v. Fagraeus, 1948; Leduc, Coons & Connally, 1953; Ehrich, Drabkin & Forman, 1949). Most supporters of the plasma cell theory, however, are agreed that the mature plasma cell is not itself an active antibody producer but is rather the morphological end-stage in the life history of the antibody-producing cell. Fagraeus (1948) regards the plasma cell as a modified reticulum cell, and advances good evidence for the idea that the intermediate stages (transitional cell and immature plasma cell) are the actual producers of antibody.

The 'large lymphoid cell' of the present study resembles the transitional cell and the immature plasma cell of Fagraeus (1948), and the 'lymphoblast' of 'acute

splenic tumour' described by Rich, Lewis & Wintrobe (1939). Our work does not definitely exclude the mature plasma cells from some part in the mechanism of antibody production, since they are certainly more numerous than normal in the medullary cords in homografted animals. They are also quite abundant in auto-grafted animals, however, and there is no doubt that the cellular response of the node to a skin homograft is largely cortical and involves predominantly the 'large lymphoid cells'. Despite their close resemblance to the immature plasma cells of Fagraeus (1948) they were never seen to transform into mature plasma cells within the cortex. It is for this reason that we have used the rather non-committal term, 'large lymphoid cells'. Both their origin and fate must remain the subject of further investigation.

The manner in which the large lymphoid cells exert their destructive influence on the skin homograft is obscure. They might conceivably act in one or both of two ways: (1) By releasing antibody into the efferent lymph and thence to the graft by way of the blood. (2) The cells themselves might leave the node and travel to the graft by a similar route. Between the 4th day and the time of onset of graft destruction, large lymphoid cells are seen in the medullary sinuses, but there are too few of them to account for the very striking reduction in their numbers in the cortex, when graft destruction has progressed for 2 or more days. Moreover, large lymphoid cells are not seen in the graft itself at any stage. There are present in the graft many small lymphocytes and mature plasma cells, but the work of Darcy (1949) on submandibular gland homografts indicates that the plasma cells develop locally within the graft.

#### SUMMARY AND CONCLUSIONS

1. The changes in weight and in cellular composition of lymph nodes and spleen have been studied in rabbits receiving an autograft or a homograft of skin on one ear.
2. The increase in weight of the first regional node on the operated side is considerably greater in homografted than in autografted animals.
3. The increase in spleen weight is only slightly greater in homografted than in autografted animals.
4. Following homografting, there develops in the first regional lymph node of the operated side a characteristic cellular response. This consists of a great accumulation of 'large lymphoid cells' in the cortex and, to a lesser extent, in the medullary cords.
5. The functional lymph drainage of the pinna has been shown to be entirely to the ipsilateral nodes. The absence of the response in the contralateral nodes establishes that the 'homograft factor' affects the recipient principally by way of the lymphatics, rather than via the blood stream.
6. This conclusion is confirmed by the absence of any demonstrable histological response in the spleen.
7. The weak response seen in node II of the operated side indicates that little of the 'homograft factor' passes the first regional node.
8. The cellular response wanes rapidly with the onset of graft destruction, and within 2 days the node is largely depleted of pyroninophilic elements.
9. It is suggested that the 'large lymphoid cell' plays an active part in the mechanism of homograft destruction.

We would like to thank Prof. G. M. Wyburn for criticism of the manuscript and Mr G. Marshall and Mr J. Byars for technical assistance. Part of the expense was defrayed by a grant from the Medical Research Council, which is gratefully acknowledged.

## REFERENCES

- BILLINGHAM, R. E., BRENT, L. & MEDAWAR, P. B. (1953). Actively acquired tolerance of foreign cells. *Nature, Lond.*, **172**, 603-612.
- BILLINGHAM, R. E., BRENT, L. & MEDAWAR, P. B. (1954). Quantitative studies on tissue transplantation immunity. II. The origin, strength and duration of actively and adoptively acquired immunity. *Proc. Roy. Soc. B* **143**, 58-80.
- BILLINGHAM, R. E. & SPARROW, E. M. (1954). Studies on the nature of immunity to homologous grafted skin, with special reference to the use of pure epidermal grafts. *J. exp. Biol.* **31**, 16-39.
- DARCY, D. A. (1949). Plasma cells in the reaction against rabbit tissue homografts. *Nature, Lond.*, **163**, 98-99.
- DOUGHERTY, T. P. & WHITE, A. (1947). An evaluation of alterations produced in lymphoid tissue by pituitary-adrenal cortical secretion. *J. Lab. clin. Med.* **32**, 584-605.
- EHRICH, W. E. (1946). The role of the lymphocyte in the circulation of the lymph. *Ann. N.Y. Acad. Sci.* **46**, 823-857.
- EHRICH, W. E., DRABKIN, D. L. & FORMAN, C. (1949). Nucleic acids and the production of antibody by plasma cells. *J. exp. Med.* **90**, 157-168.
- EHRICH, W. E., HARRIS, T. N. & MERTENS, E. (1946). The absence of antibody in the macrophages during maximum antibody formation. *J. exp. Med.* **83**, 373-381.
- FAGRAEUS, A. (1948). Antibody production in relation to the development of plasma cells. *Acta med. scand. (Suppl.)*, **204**, 5-122.
- GALLONE, L., RADICI, G. & RIQUEIR, G. (1952). La reazione dei linfogangli regionali agli omoi-HARRIS, T. N., GRIMM, E., MERTENS, E. & EHRICH, W. H. (1945). The role of the lymphocyte in antibody formation. *J. exp. Med.* **81**, 73-83.
- nesti de pelle. *Arch. atti Soc. Ital. Chir.* **2**, 329-330.
- HARRIS, T. N. & HARRIS, S. (1948). Histologic evidence for the synthesis of protein in lymphocytes following parenteral injection of antigen. *Proc. Soc. exp. Biol., N.Y.*, **69**, 18-19.
- HARRIS, T. N. & HARRIS, S. (1949). Histochemical changes in lymphocytes during the production of antibodies in lymph nodes of rabbits. *J. exp. Med.* **90**, 169-180.
- KROHN, P. L. & ZUCKERMAN, A. (1954). The effect of splenectomy on the survival of skin homografts in rabbits and on the response to cortisone. *Brit. J. exp. Path.* **35**, 223-226.
- LEDUC, E. H., COONS, A. H. & CONNALLY, J. M. (1953). Immuno-histochemical localization of antibodies in the plasma cell and its progenitors. *Anat. Rec.* **117**, 580 (Abstr.).
- MCMASTER, P. D. (1953). Sites of antibody formation. In *The nature and significance of the Antibody Response* (A. M. Pappenheimer, editor). N.Y.: Columbia University Press, 13-45.
- MCMASTER, P. D. & HUDACK, S. S. (1935). The formation of agglutinins within lymph nodes. *J. exp. Med.* **61**, 783-807.
- MEDAWAR, P. B. (1945). A second study of the behaviour and fate of skin homografts in rabbits. *J. Anat., Lond.*, **79**, 157-176.
- MEDAWAR, P. B. (1954). General problems of immunity. In *Preservation and Transplantation of Normal Tissues*, pp. 1-20. Wolstenholme, G. E. W. and Cameron, M. P., editors. London: J. and A. Churchill.
- MITCHISON, N. A. (1954). Passive transfer of transplantation immunity. *Proc. Roy. Soc. B*, **142**, 72-87.
- RICH, A. R., LEWIS, M. R. & WINTROBE, M. M. (1939). The activity of the lymphocyte in the body's reaction to foreign proteins, as established by the identification of the acute splenic tumor cell. *Johns Hopk. Hosp. Bull.* **65**, 311-327.
- SABIN, F. R. (1939). Cellular reactions to a dye-protein, with a concept of the mechanism of antibody formation. *J. Exp. Med.* **70**, 67-82.



## EXPLANATION OF PLATES

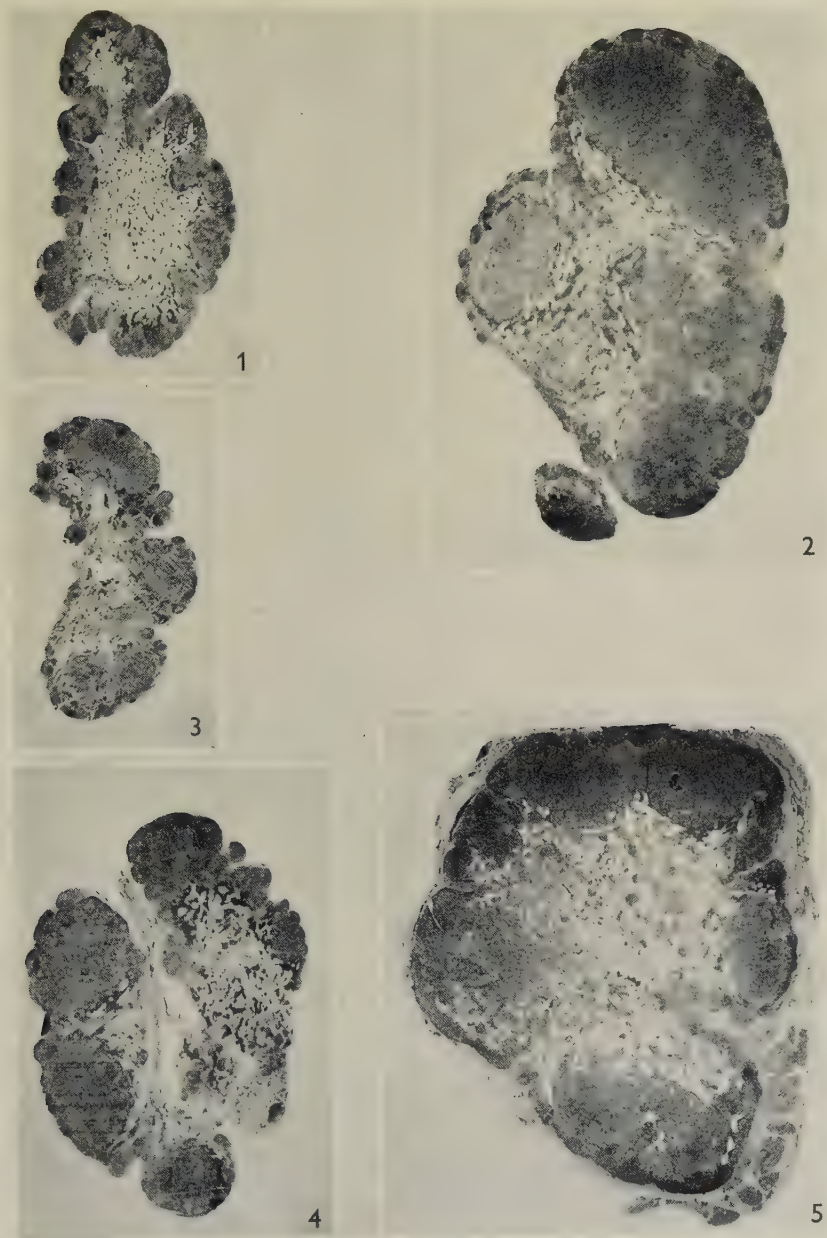
All figures are of node I, the first regional node receiving lymph from the pinna. Methyl-green-pyronin stain.

PLATE 1. All  $\times 6.25$ 

- Fig. 1. Node from unoperated control (R 323).  
Fig. 2. Node from operated side of R 319, 4-day homograft. Note great enlargement of tertiary cortical nodules. Germinal centres are small and subcapsular.  
Fig. 3. Node from unoperated side, R 319, 4-day homograft.  
Fig. 4. Node from operated side, R 321, 4-day autograft. Note moderate enlargement of tertiary cortical nodules and of the medullary cords, which are more darkly stained (pyroninophilic) than normal.  
Fig. 5. Node from operated side, L 264, 10-day homograft. Tertiary cortical nodules still enlarged, germinal centres small and subcapsular. The medullary cords are also still enlarged, but in places are very palely stained, and under high power show marked cellular depletion.

PLATE 2. All  $\times 600$  approx. Ektachrome. Each field is part of a tertiary cortical nodule

- Fig. 6. Node from operated side of R 319, 4-day homograft.  
Fig. 7. Node from operated side of R 350, 6-day homograft, early graft breakdown. Shows abundant large lymphoid cells of tertiary cortical nodule.  
Fig. 8. Node from unoperated side of R 350. Large lymphoid cells absent.  
Fig. 9. Node from operated side, L 264, 10-day homograft; graft breakdown advanced. Illustrates the disappearance of large lymphoid cells.  
Fig. 10. Node from operated side, L 292, 5-day autograft. Large lymphoid cells absent.

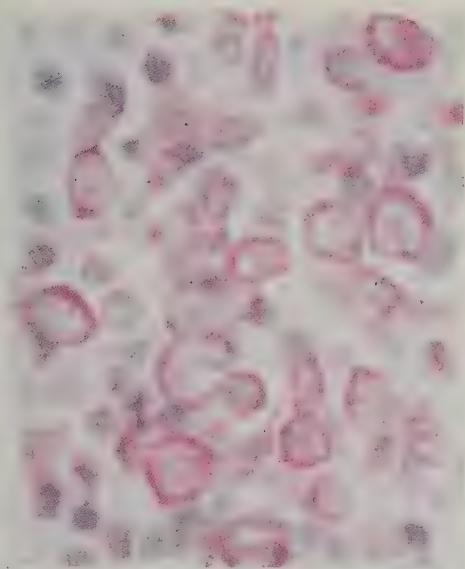


SCOTHORNE AND MCGREGOR—SKIN HOMOGRAFTING IN THE RABBIT

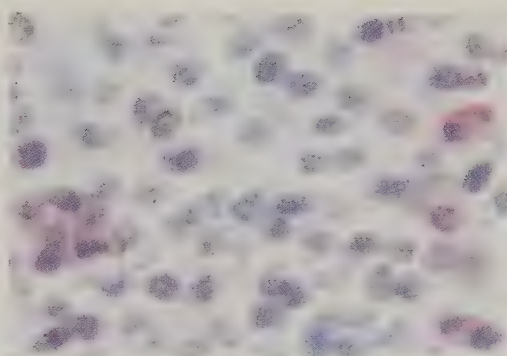
(Facing p. 292)



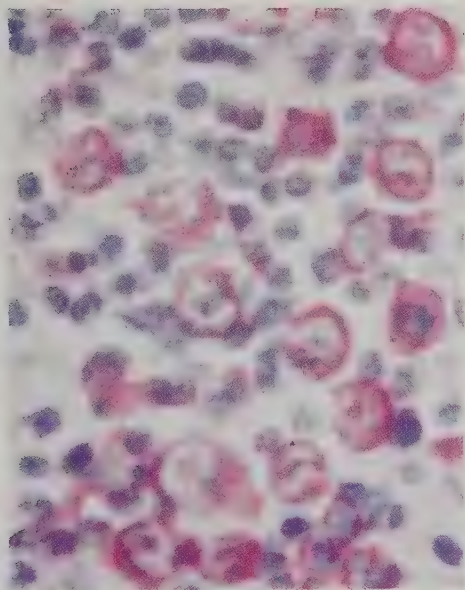




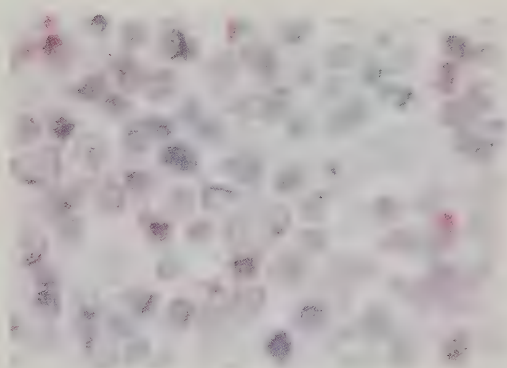
6



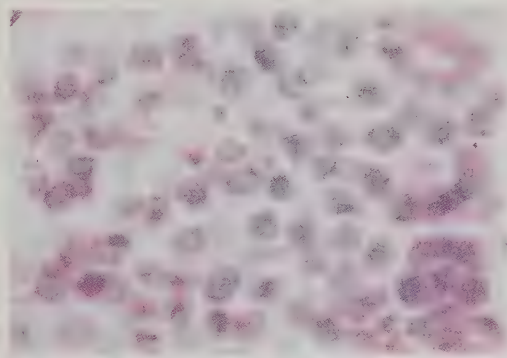
8



7



9



10



# ADRENO-CORTICAL HISTOGENESIS IN THE RAT: WITH OBSERVATIONS ON LIPID AND ASCORBIC ACID DISTRIBUTION

BY J. D. LEVER

*Department of Anatomy, University of Cambridge*

## INTRODUCTION

A derivation of the adreno-cortical anlage by cell migration from a mesothelial thickening in the posterior coelomic wall has long been described; in man by Zuckerkandl (1912), Wieman (1920), Hett (1925) and Keene & Hewer (1927); in the rat by Pankratz (1931); in the guinea-pig by Harman & Derbyshire (1932); and in the mouse by Waring (1936). According to Keene & Hewer (1927) and Uotila (1940), in the human adrenal a second proliferation of the coelomic lining takes place in embryos of about 12 mm., the cells so formed migrating dorsally to surround the first anlage and become the permanent cortex: a mesothelial derivation for the permanent or adult cortex has been claimed by Davies (1938) in the cat, and Harman & Derbyshire (1932) in the guinea-pig. On the other hand, Gruenwald (1946), describing the human permanent cortex as mesenchymal in origin, did not believe a hard and fast division was possible between adult and foetal cortical anlagen. Much earlier Pankratz (1931) made no distinction between foetal and adult cortices in describing the developing adrenal in the rat.

Opinion is divided on the question of when individual cortical zones are differentiated. Thus Jackson (1919), in the rat, claimed that the zonae glomerulosa, fasciculata and reticularis were all present at birth; and Harman & Derbyshire (1932) described all three zones in the guinea-pig gland at 1 day. Keene & Hewer (1927) described a z. glomerulosa at birth in the human adrenal and claimed that the fasciculata had differentiated by the 3rd week and the reticularis by the 14th. In their accounts of the rat gland, Howard (1938) and Mitchell (1948) are essentially in agreement: according to them the glomerulosa appears within the 1st week, the fasciculata within the 2nd and the reticularis during the 3rd week. In addition, Mitchell (1948) and Cater & Lever (1954) described the appearance, at about 3 days, of a z. intermedia between glomerulosa and fasciculata in the rat adrenal.

Keene & Hewer (1927), in the human gland, reported the appearance of sudanophile lipids in both foetal and adult cortices at about the 6th month of intrauterine life: at term lipids were absent from the foetal cortex. More recently, Dawson (1953), in the chick embryo at the 11th day, reported definitely positive reactions on adreno-cortical cells with the Sudan stains and with the Schultz, Plasmal and Ashbel-Seligman tests: he also demonstrated (by the acid silver nitrate method) the first appearance of cortical ascorbic acid at the 11th day of incubation.

Bourne (1933), after acid silver nitrate impregnation, reported an intracellular deposition of silver granules in both cortex and medulla of the adult cat, guinea-pig,



rat and mouse adrenals; while Gough & Zilva (1933) claimed that impregnation of medullary cells, though normally present in the canine and human glands, was not detectable in the rat medulla. Following stress Bourne (1934) noted that the medullary exceeded the cortical ascorbic acid content in the kangaroo, rabbit, guinea-pig and cat adrenals. In spite of these observations Giroud & Leblond (1935) and Leblond & Gardner (1938) clearly regarded the adrenal distribution of demonstrable (by acid silver impregnation) ascorbic acid as purely cortical: they even claimed that in the embryonic sheep and rat adrenals cortical and medullary tissues could be visually separated because of the negative medullary and positive cortical reactions to silver impregnation. Subsequently, Barnett & Bourne (1942) described a positive (acid) silver reduction by both cortical and medullary cells of the 14-day chick embryo adrenal; the medullary silver granules were larger and more numerous than those in the cortex.

An investigation of the appearance and distribution of sudanophil lipid and ascorbic acid in the developing rat adrenal is reported in this paper.

#### MATERIALS AND METHODS

Throughout the investigation rats of the white Wistar strain were used.

(1) *Embryonic adrenals.* Animals were left together until mating had occurred; fulfilment of this was assessed by daily vaginal smears, and pregnancy was dated from the time of demonstration of spermatozoa in the smears. Rats were sacrificed in pairs when 8, 10, 12, 13, 14, 15, 16, 17, 18, 19 and 20 days pregnant and embryos from each were sufficiently dissected to allow of free access of fixatives etc. to the adrenal rudiment. Whole embryos or requisite portions of them (according to size) were treated as follows: (a) fixed in Baker's calcium-formol saline, gelatine-embedded and then freeze cut at  $25\mu$  for staining with Sudan black, or (b) Susa-fixed, paraffin-embedded and sectioned at  $6-7\mu$  for haematoxylin and eosin staining; or (c) stained for ascorbic acid by the method of Deane & Morse (1949), employing a saturated alcoholic solution of silver nitrate for impregnation (45 min.).

(2) *Neonatal adrenals.* A total of twenty-two young rats were sacrificed in pairs as follows: at birth, and at daily intervals up to 10 days after birth. Adrenals were treated as in 1 (a), (b) and (c), one gland from each animal being bisected to provide sufficient material for all three procedures.

#### RESULTS

##### *The 12-day (7 mm.) rat embryo adrenal*

The anlage of the rat adrenal cortex first appears in the 7 mm. (12 day) embryo (Pl. 1, fig. 1). It extends cranially in the posterior wall of the pleuro-peritoneal canals as far as the level of the tracheal bifurcation and caudally down the posterior coelomic wall on either side of the root of the dorsal mesentery. The anlage comprises a longitudinal thickening of the mesothelium, consisting of columnar cells with elongated vesicular nuclei (Pl. 1, fig. 1). Immediately deep to this thickening or ridge is a small collection of irregularly disposed and faintly eosinophilic cells which would appear to be derived from the overlying columnar cells. At this stage it is probable that the rudiment described is common to both adrenal and gonad. In

the 12 mm. (14 day) embryo it is apparent that the adrenal anlage, now a well-defined posterior body wall structure, is medial to the gonad and mesonephros, and lateral to the sympatho-chromaffin cell anlage of the sympathetic chain and adrenal medulla. The cephalo-caudad extent of the adrenal is approximately from the pleuro-peritoneal opening to the level of the cardiac orifice of the stomach (Pl. 1, fig. 2); this narrowing of the longitudinal extent of the anlage is most likely due to processes of differential growth. The adrenal cortical rudiment now consists of a collection of large acidophilic cells some of which exhibit mitotic figures. It is separated from both the overlying columnar mesothelium and also the gonad and mesonephros, by what appears to be a loose capsule of stromal cells disposed circumferentially around it (Pl. 1, fig. 3). In this and succeeding stages there were no obvious cell contributions from the overlying mesothelium, which in time becomes progressively flatter in the neighbourhood of the adrenal, but remains elevated and columnar over the gonad. It would appear therefore that the further development of the anlage depends upon its own powers of cell division (which are apparent) and probably, and to an indefinable extent, upon contributions from the surrounding stromal cells (Pl. 1, fig. 3).

*The 16- to 18-day (18-24 mm.) rat embryo adrenal*

In 18 mm. embryos (16-17 days), the adrenal is a well-defined body bulging ventrally from the posterior abdominal wall and lying cephalo-posterior to the metanephros. It possesses a well-marked capsule, the cells of which are however loosely arranged medially where the anlage is in contact with the sympatho-chromaffin tissue lying either side the aorta (Pl. 1, fig. 4). An invasion of the cortical anlage by sympatho-chromaffin cell islands migrating centripetally into the gland is well under way at the 18 mm. stage. The adrenal anlage, at an earlier stage (12 mm.) a close medial relation of the gonad, now lies well above this and its associated mesonephric rudiments. Also at the 18 mm. stage (Pl. 1, fig. 5) some differentiation of the cortical anlage is apparent. Centrally the cortical cells are large and deeply eosinophilic with a pale staining nucleus, and are arranged in loose columns separated by capillary spaces or islands of sympatho-chromaffin tissue; immediately deep to the capsule and in many places indistinguishable from it, is a region of small darker-staining cells. It is impossible exactly to demarcate these two regions; in some places they are distinct, while in others they merge into one another. In the adrenal of the 18 mm. embryo mitotic figures are numerous in the capsule and the outer of the two cortical regions. At approximately the 18 mm. stage discrete sudanophile lipid droplets (additional to a general background coloration with Sudan black) are first discernible, particularly in the outer cortical layers (Pl. 2, fig. 6): at the same time the presence of ascorbic acid in the medullary islands is indicated by the present method as a series of fine intracellular silver granules (P. 1, fig. 5).

By the 24 mm. (18-day) stage further cortical differentiation is apparent. Beneath the capsule there is a layer, 1 to 3 cells thick, containing numerous mitotic figures; the cells of the layer have a small amount of basophilic cytoplasm containing a few fine lipid droplets. The remainder of the adrenal cortex consists of a zone of larger, paler cells with a spongy cytoplasm, arranged in a roughly fasciculate manner

(Pl. 2, fig. 7) and enclosing a central mass of large eosinophilic cells, which is the foetal cortex. At this stage mitoses are present not only in the capsule and underlying zone of small basophilic cells, but also in the outer part of this embryonic fasciculate zone. The cells of the foetal cortex show a homogeneous light coloration with Sudan stains but are devoid of recognizable lipid droplets, while the fasciculate cells contain numerous lipid droplets. Ascorbic acid is detectable in the islands of medullary tissue which lie scattered amongst the cortical cells towards the centre of the gland.

*The rat embryo adrenal from 20 days (35–40 mm. according to litter size)  
until full term*

In the 20-day embryo (35–40 mm.) the cortex is further organized, and the medulla is tending to be loosely arranged about the large central blood spaces. The outer zone of dark-staining cells beneath the capsule is broader but is not yet clearly a zona glomerulosa. The zone immediately within it shows an outer part of vacuolated (spongicyte) cells arranged in a rough fasciculate pattern and an inner part consisting of cell columns loosely disposed between blood spaces. Unlike the human foetal cortex or the mouse X zone, the rat foetal cortex, referred to by Howard (1938) as the 'juvenile cortex', shows no signs of cell degeneration and is probably incorporated into the innermost layers of the differentiating adult cortex. Possibly in support of this contention of disappearance of the foetal cortex by incorporation is the fact that the mitotic distribution, although maximal in the outer cortical layers now extends to the deeper cortex: a feature of earlier preparations (18 mm.) was that the foetal cortical cells toward the gland centre did not show mitosis. By the 20th day the silver reaction for ascorbic acid is positive not only in the medullary islands, but to a less marked extent (finer and fewer granules) throughout the cortex (Pl. 2, fig. 8). Lipids, sparse in the immediately subcapsular cortical layers, are present in quantity in the rest of the cortex (Pl. 2, fig. 9).

At birth the z. glomerulosa is discernible as a region of small basophilic cells grouped in oval or spherical clusters and the outer fasciculata is already arranged in radial cell columns: the deep cortex is disposed in irregular cords around wide capillary blood spaces. The medulla is a loose central collection of cell islands still interdigitating irregularly with the cortex. Ascorbic acid is now but patchily present in the medulla, some of the cell islands being completely devoid of silver granules; while in the cortex most of the silver deposit is found lining blood vessel walls (Pl. 2, fig. 10), a fact which may be construed as evidence of active cortical secretion (Deane & Morse, 1949; Cater & Lever, 1954).

*The neonatal rat adrenal*

At the 3rd day the z. intermedia (Nicander, 1952; Lever, 1954*a*) is apparent immediately deep to the glomerulosa as a narrow band in which the number of cells per unit area is increased over neighbouring zones (Cater & Lever, 1954): at its appearance in both sexes, the intermedia stands out as a lipid-free zone between the lipid-containing glomerulosa and fasciculata in Sudan stained preparations. By the 7th day the cell arrangement in the outer z. fasciculata is more definitely in radial



columns; and the reticularis emerges as an entity separate from the inner fasciculata at about the 3rd week (Lever, 1954*b*).

Until approximately the 5th day there is reduction of acid silver nitrate by some of the medullary cells, but after this the reaction, while continuing positive in the cortex, is negative for the medulla (Pl. 2, fig. 11).

#### DISCUSSION

From this investigation it would appear that the rat adrenal cortex develops from two distinct sources: the initial anlage is derived by cell proliferation from coelomic mesothelium which then makes no further contribution to the rudiment. Subsequently the cortex grows as a result of its own mitoses and cell additions from surrounding mesenchymal elements. It is difficult in the rat to distinguish between adult and foetal cortices. The latter does not undergo degeneration as in the human, cat and mouse adrenals and is therefore not so distinctive; it is probable that it ultimately gives rise to the *z. reticularis* in the 3-week old rat.

The question as to when the cortical zones should be designated *glomerulosa*, *fasciculata* and *reticularis* does not permit of easy answer. If the shape of cell groupings is accepted as a satisfactory criterion for zone classification then the present work suggests that while the *z. glomerulosa* is present at about the time of birth, the outer *fasciculata* and its cells begin to contain discrete lipid droplets at approximately the 18th day of intrauterine life (24 mm.); the format (of radial cell columns) of the *z. fasciculata* is greatly consolidated post-natally but it can be identified before birth.

Deane & Morse (1949) believe that ascorbic acid is present in all cortical cells capable of producing lipids. Thus they reported the coincident reappearance of demonstrable intracellular lipid and ascorbic acid during cortical regeneration following adrenal enucleation in rats. After Sayers, Sayers, Lang & Long (1945) had proved a relationship of ascorbic acid to adrenal steroid output by demonstrating a coincident reduction of adrenal ascorbic acid and cholesterol following stress, Bourne (1949) suggested a water-solubilizing action for ascorbic acid when linked as a side chain to the insoluble steroids. In the present investigations on the rat embryo adrenal, although discrete intracellular lipid droplets were present by the 16th–18th day, cortical ascorbic acid was not demonstrated until just prior to birth on the 20th day.

Following his observation that ascorbic acid was only demonstrable (by silver reduction) after stress in the rat adrenal medulla, Bourne (1949) suggested that the vitamin was normally present in a reversibly oxidized form in the medulla. In support of this Bahn & Glick (1954), by microspectrophotometric investigation, found all the dehydroascorbic and diketogulonic acids to be exclusively in the medulla.

In the embryonic adrenal the ability to reduce acid silver nitrate is possessed by medullary before cortical cells and is lost by the former at about 6 days after birth. The loss of the medullary reaction probably represents a transformation of ascorbic acid to its reversibly oxidized form. This change over, although complete by about the 6th post-natal day, probably commences at about birth, since at and from then a large proportion of medullary cells are unstained by silver. An event commencing

at birth, proceeding for some 5 or 6 days, and which might have a possible bearing on the problem, is the centralization of the medulla as a body discrete from the cortex.

#### SUMMARY

1. Adrenals from neonatal rats, and rat embryos of known age, were histologically examined and their ascorbic acid and sudanophile lipid distributions were investigated.

2. At the 12th day of intrauterine life the initial cortical anlage is derived by dorsal migration of cells from a linear proliferation of the mesothelium in the posterior wall of the pleuro-peritoneal canal and along the coelomic wall either side of the dorsal mesentery. Subsequent growth of the cortical anlage is by its own mitosis and by addition from surrounding mesenchyme.

3. It is difficult to distinguish between adult and foetal cortices; the latter does not degenerate but probably gives rise to the z. reticularis at about the 3rd post-natal week.

4. Although the z. glomerulosa is not recognizable until birth, a fasciculate cell arrangement is apparent in the position of outer z. fasciculata by the 18th day of intrauterine life.

5. Discrete lipid droplets appear in the outer layers of the adrenal cortex of the 16- to 17-day embryo: there is a coincident appearance of ascorbic acid in the medullary islands: cortical ascorbic acid does not appear until just prior to birth at the 20th day of intrauterine life. A marked reduction of (demonstrable) ascorbic acid in the medulla at birth continues, and 6 days later it is demonstrable only in the cortex. This reversal in the distributions of detectable cortical and medullary ascorbic acid is discussed.

I am greatly indebted to Prof. J. D. Boyd for his encouragement and advice. I also wish to thank Mr R. Smith for technical assistance; and Mr T. R. L. Brooks and Miss J. P. Shanks for the photographs.

#### REFERENCES

- BAHN, R. C. & GLICK, D. (1954). The quantitative histological distribution of ascorbic acid in the adrenal gland of the rat and monkey. *J. Histochem. Cytochem.* **2**, 103-109.
- BARNETT, S. A. & BOURNE, G. (1942). Distribution of ascorbic acid in cells and tissues of the developing chick. *Quart. J. micr. Sci.* **83**, 259-298.
- BOURNE, G. (1933). The staining of vitamin C in the adrenal glands. *Aust. J. exp. Biol. med. Sci.* **11**, 261-267.
- BOURNE, G. (1934). Vitamin C. *Med. J. Aust.* pp. 339-343.
- BOURNE, G. (1949). *The Mammalian Adrenal*, p. 194. Oxford: Clarendon Press.
- CATER, D. B. & LEVER, J. D. (1954). The zona intermedia of the adrenal cortex; a correlation of possible functional significance with development, morphology and histochemistry. *J. Anat., Lond.*, **88**, 437-454.
- DAVIES, S. (1938). The development of the adrenal gland of the cat. *Quart. J. micr. Sci.* **80**, 81-98.
- DAWSON, A. B. (1953). Histochemical evidence of early differentiation of the suprarenal gland of the chick. *J. Morph.* **92**, 579-589.
- DEANE, HELEN W. & MORSE, ANNA (1949). The cytological distribution of ascorbic acid in the adrenal cortex of the rat under normal and experimental conditions. *Anat. Rec.* **100**, 127-136.
- GIROUD, A. & LEBLOND, C. P. (1935). Localisations électives de l'acide ascorbique ou vitamine C. *Arch. Anat. micr.* **31**, 111-142.

- GOUGH, J. & ZILVA, S. S. (1933). The silver nitrate staining reaction for ascorbic acid in the adrenal, pituitary and ovary, of various species of animals. *Biochem. J.* **27**, 1279-1286.
- GRUENWALD, P. (1946). Embryonic and postnatal development of the adrenal cortex, particularly the z. glomerulosa and accessory nodules. *Anat. Rec.* **95**, 391-415.
- HARMAN, MARY T. & DERBYSHIRE, R. C. (1932). The development of the suprarenal glands in the guinea pig. *Amer. J. Anat.* **49**, 335-347.
- HETT, J. (1925). Ein Beitrag zur Histogenese der menschlichen Nebenniere. *Z. mikr.-anat. Forsch.* **3**, 179-282.
- HOWARD, EVELYN (1938). The representation of the adrenal X zone in rats in the light of observations on X zone variability in mice. *Amer. J. Anat.* **62**, 351-367.
- JACKSON, C. M. (1919). The post-natal development of the suprarenal gland, and the effects of inanition upon its growth and structure in the albino rat. *Amer. J. Anat.* **25**, 221-281.
- KEENE, MARY F. L. & HEWER, E. E. (1927). Observations on the development of the human suprarenal gland. *J. Anat., Lond.*, **61**, 302-324.
- LEBLOND, C. P. & GARDNER, W. U. (1938). Distribution of vitamin C in the adrenal gland of the mouse with reference to the nature of the X zone. *Anat. Rec.* **72**, 119-127.
- LEVER, J. D. (1954*a*). Vascular zoning in the adrenal cortex of the normal and hypophysectomised rat with observations on the distribution of lipids. *J. Endocrin.* **10**, 133-146.
- LEVER, J. D. (1954*b*). Anatomy of the Adrenal Cortex. Thesis submitted for M.D. Camb. Univ., pp. 95-99.
- MITCHELL, R. M. (1948). Histological changes and mitotic activity in the rat adrenal during post-natal development. *Anat. Rec.* **101**, 161-181.
- NICANDER, L. (1952). Histological and histochemical studies on the adrenal cortex of domestic and laboratory animals. *Acta Anat.* **14**, Suppl.
- PANKRATZ, D. S. (1931). The development of the suprarenal gland in the albino rat with a consideration of its possible relation to the origin of foetal movements. *Anat. Rec.* **49**, 31-49.
- SAYERS, G., SAYERS, MARION A., LANG, T. & LONG, C. N. H. (1945). The cholesterol and ascorbic acid content of the adrenal, liver, brain and plasma, following haemorrhage. *Endocrinology*, **37**, 96-110.
- UOTILA, U. U. (1940). The early embryological development of the foetal and permanent adrenal cortex in man. *Anat. Rec.* **76**, 183-195.
- WARING, H. (1936). The development of the adrenal gland of the mouse. *Quart. J. micr. Sci.* **78**, 329-365.
- WIEMAN, H. L. (1920). Observations in connection with the early development of the human suprarenal gland. *Anat. Rec.* **19**, 269-279.
- ZUCKERKANDL, E. (1912). The development of the chromaffin organs and of the suprarenal glands. Ch. 15, in KEIBEL, F. & MALL, F. P., *Manual of Human Embryology*, vol. II, pp. 157-179.

## EXPLANATION OF PLATES

## PLATE 1

- Fig. 1. From a transverse section of a 12-day rat embryo: the adreno-genital rudiment (*a*) appears as a proliferation of the mesothelium of the pleuro-peritoneal canal: stained haematoxylin and eosin (H. & E.); 6 $\mu$ ;  $\times 406$ .
- Fig. 2. Paramedian sagittal section of a 14-day rat embryo. The adreno-cortical anlage in the posterior coelomic wall is roughly diamond-shaped in outline (*a*); dorsal to it a disconnected chain of dark sympatho-chromaffin tissue (*s*); ventrally and below, part of the gonad (*g*), then the dorsal mesentery with lesser sac and stomach (*v*); ventrally and above, the pleuro-peritoneal opening and lung bud (*l*); stained H. & E.; 6 $\mu$ ;  $\times 406$ .
- Fig. 3. Transverse section of 14-day rat embryo. The adreno-cortical anlage consists of large eosinophilic cells showing mitoses: it is separated from the mesonephros (fig. bottom left) and the coelomic mesothelium by a loose stromal capsule: stained H. & E.; 6 $\mu$ ;  $\times 406$ .
- Fig. 4. Transverse section of 16-day rat embryo. The adrenal (*a*) is now a discrete organ bulging ventrally towards the coelomic cavity. It has a well-marked fibro-cellular capsule except medially where an invasion by sympatho-chromaffin cells (*s*) is taking place; dark islands of sympatho-chromaffin tissue are seen deeper in the gland: stained H. & E.; 6 $\mu$ ;  $\times 406$ .



Fig. 5. Seventeen-day rat embryo adrenal stained for ascorbic acid by an acid silver nitrate method and counterstained with haematoxylin and eosin. Ascorbic acid detected by a black granular silver deposit is only present in medullary cell islands, seen interspersed between cortical cells towards the gland centre. There is evidence of differentiation of a narrow subcapsular zone of small dark cells:  $6\mu$ ;  $\times 406$ .

## PLATE 2

Fig. 6. Frozen section of an 18-day rat embryo adrenal showing lipid distribution. An immediately subcapsular zone contains sparse fine droplets; within this is a zone of heavier staining possibly corresponding to the fasciculate cells in fig. 7; within this is a zone of fainter coloration without definitive droplets (this is likely the foetal cortex); the unstained islands are sympatho-chromaffin tissue: stained Sudan black;  $25\mu$ ;  $\times 120$ .

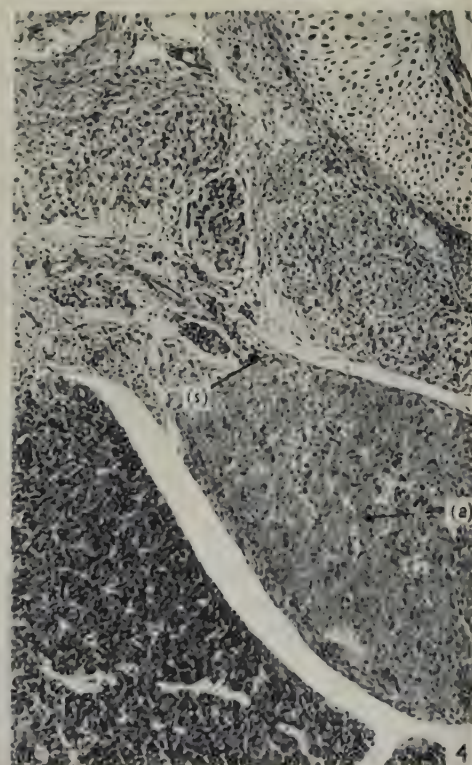
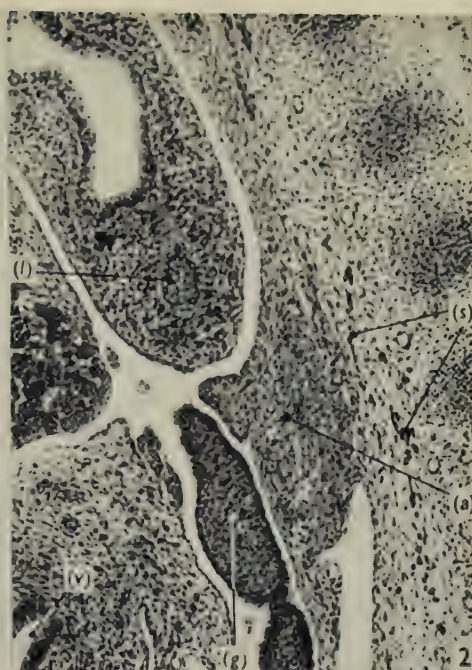
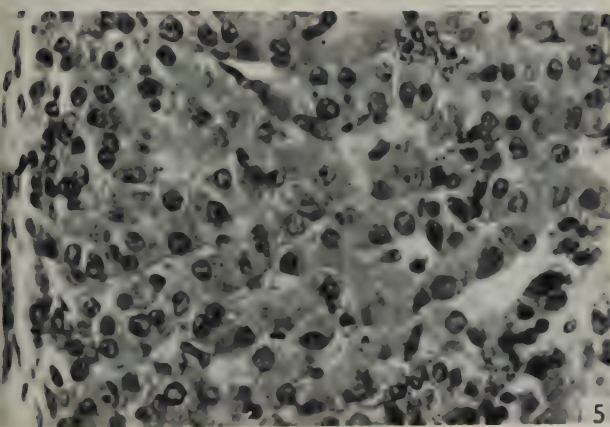
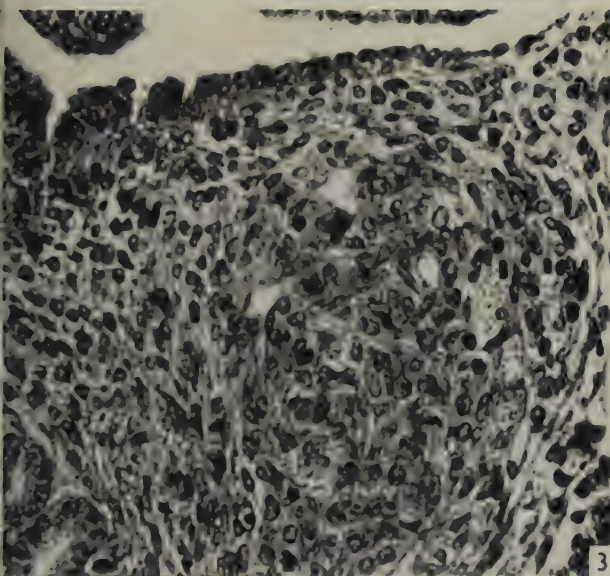
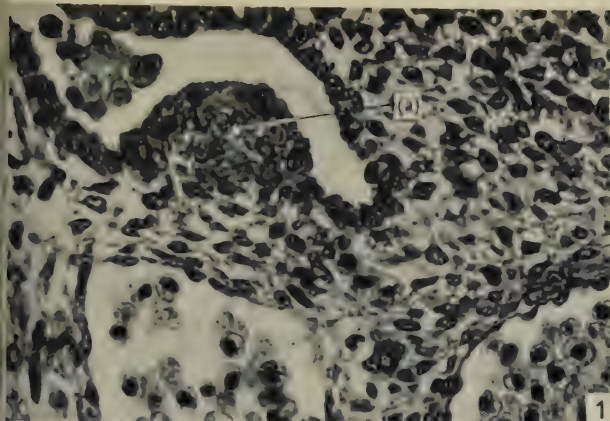
Fig. 7. Eighteen-day rat embryo adrenal. There is not a clear distinction between adult cortex and capsular cells: cells in definite fasciculate arrangement are seen extending inwards for a short distance: a nerve bundle with a small group of sympatho-chromaffin cells (*s*) in advance of it is seen extending into the gland: stained H. & E.;  $6\mu$ ;  $\times 406$ .

Fig. 8. Twenty-day rat embryo adrenal stained as in fig. 5. Silver granules indicative of ascorbic acid, are now widely present in the cortical cells and in greater concentration in the medullary islands (one of which lies near bottom right of print):  $6\mu$ ;  $\times 406$ .

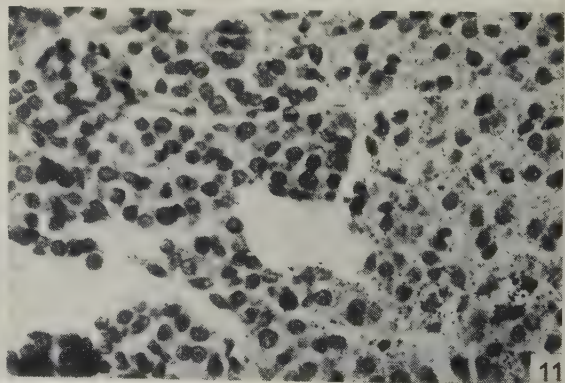
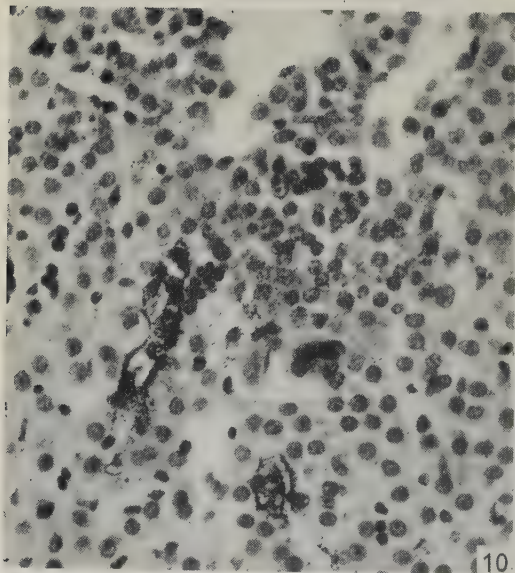
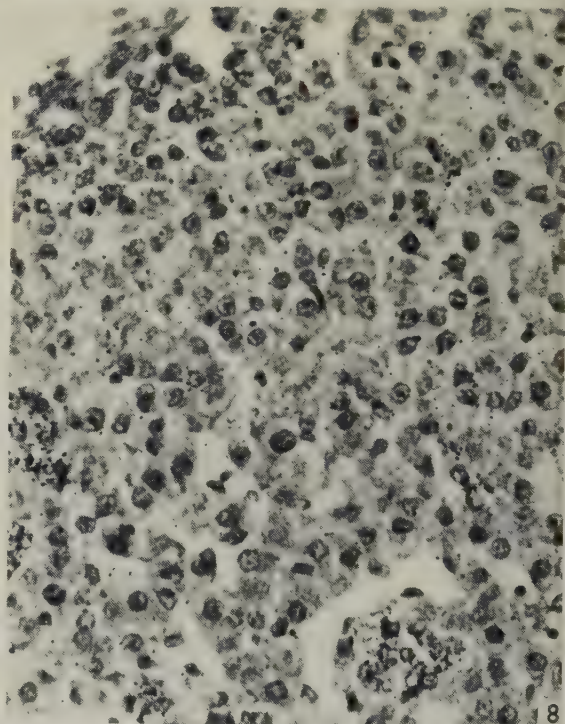
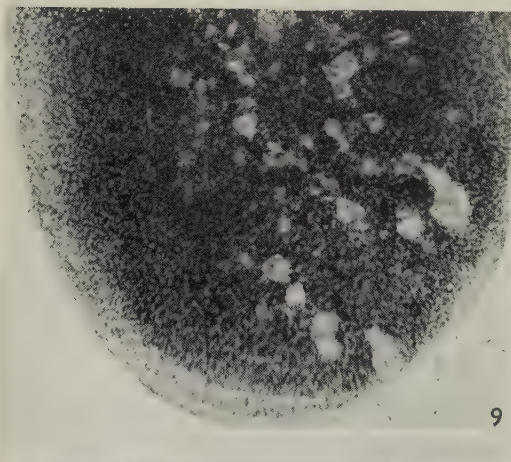
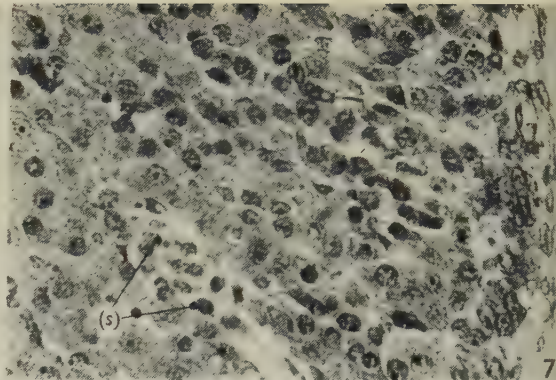
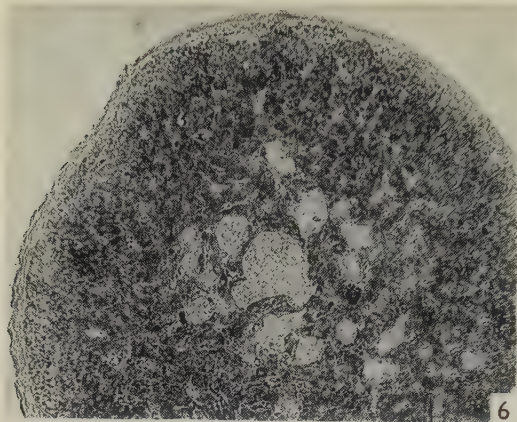
Fig. 9. Twenty-day rat embryo adrenal stained for lipid as in fig. 6. The entire cortex now contains lipid which however is sparse in the glomerulosa: medullary tissue is unstained:  $25\mu$ ;  $\times 91$ .

Fig. 10. Rat adrenal at birth stained as in figs. 5 and 8. Ascorbic acid is present in quantity in the deep cortical blood vessels and in some of the medullary tissue (an island of which is seen print centre):  $6\mu$ ;  $\times 406$ .

Fig. 11. Six-day old rat adrenal stained as in figs. 5, 8 and 10: ascorbic acid is now only demonstrable in cortex (print right):  $6\mu$ ;  $\times 406$ .









# HYPOTHALAMIC NEUROSECRETION IN THE DOG AND CAT, WITH PARTICULAR REFERENCE TO THE IDENTIFICATION OF NEUROSECRETORY MATERIAL WITH POSTERIOR LOBE HORMONE

By J. C. SLOPER

*Bernhard Baron Institute of Pathology, London Hospital*

The site of formation of the hormones of the posterior lobe of the pituitary has long been disputed. Thus they are held by some to be secreted by the pituicytes of the infundibular process (Gersh, 1939) and by others by the neurones of the supraoptic and paraventricular nuclei, whence they are passed along axones to the infundibular process (Scharrer & Scharrer, 1937). This second theory, although quite compatible with the known behaviour of neurones (Zuckerman, 1954) because of technical difficulties gained little credence until Bargmann (1949) showed that with Gomori's (1939) chrome-haematoxylin technique a stainable material could readily be demonstrated in mammals as well as other vertebrates, the distribution of which favoured the theory of hypothalamic secretion. This so-called 'neurosecretory material' (N.S.M.) is characteristically aggregated in the cytoplasm of the neurones of the supraoptic and paraventricular nuclei, whence it passes along their axones in the form of fibres, often beaded like 'strings of pearls'. According to Laqueur (1954), in the dog at least, there is a direct pathway from paraventricular as well as supraoptic nucleus to infundibular process. There and in the infundibular stem, large aggregations of stainable material are seen, which may be identified with Herring bodies; but in the infundibular process the greater part of this stainable material is diffusely and finely distributed, particularly at the periphery. Two remarkable claims are made for Gomori's stain, namely first that the material in the cytoplasm of the cell-body of the neurone is identical with that spreading down its axone: and second that this material is extremely limited in its distribution.

Since Bargmann's original observations much has been published in favour of hypothalamic neurosecretion (Scharrer & Scharrer, 1954), in particular by Hild (1951), who demonstrated the accumulation of N.S.M. above the cut pituitary stalk, and by Hild & Zetler (1951) who isolated oxytocic, vasopressor and antidiuretic substances from the hypothalamus as well as from the infundibular process. It is probable that these substances are present in differing proportions in the two sites, an observation which suggests either secretion at both sites, or alternatively secretion at one site, with chemical modification at the other (Vogt, 1953). The fact that there is a close similarity between the formulae of two cyclic octapeptides recently published by Du Vigneaud and his colleagues (Du Vigneaud, Ressler, Swan, Roberts, Katsoyannis & Gordon, 1953; Du Vigneaud, Lawler & Popenhoe, 1953), the one with oxytocic and the other with vasopressor-antidiuretic activity, supports the second view.

The purpose of this work is the re-examination of Bargmann's theory, with a view to its corroboration and modification in the light of these recent advances. Observations have been confined to the dog and cat. First the relative distribution of certain enzymes has been studied in the supraoptic and paraventricular nuclei as compared with the infundibular process, on the hypothesis that secretion should be accompanied by particularly marked cellular activity in one site or the other. Secondly, the specificity of the stains used for the demonstration of N.S.M. has been reassessed. Thirdly, the histochemistry of N.S.M. has been investigated. It will be shown that, although N.S.M. is claimed to be a complex and variable glycolipoprotein (Schiebler, 1951, 1952*a*), a bearer-substance, akin to thyroid colloid, and readily separable from posterior-lobe hormone (Hild & Zetler, 1952-3), this substance is more probably a protein, representing the hormone itself.

#### METHODS

Observations were made on thirty adult cats and eleven adult dogs, anaesthetized with chloroform, ether, or Nembutal, and decapitated. The techniques were applied to normal cats, and were confirmed in animals used for a variety of experiments, none of which apparently affected the morphology of the neurohypophysis. All but five of the eleven dogs had undergone a jejunocolic anastomosis at least a month previously.

Rapid dissection following the use of a band-saw allowed the immersion of hypothalamus and pituitary, within 10 min. of death, either in formol-saline (4 %), in various fat solvents (e.g. 80 % ethyl alcohol), or in a dry test-tube placed in a mixture of CO<sub>2</sub> snow and acetone. Frozen sections were normally cut unfixed at -20° C. in the Coons-Linderström-Lang cryostat (Coons & Kaplan, 1950). Sagittal sections from the pituitary, hypothalamus and adjacent brain tissue of the cat were studied for enzyme distribution. Unfixed frozen sections were readily cut at 5-15  $\mu$  in the cryostat and mounted direct on a dry slide: sections were then thawed to room temperature, becoming at this stage adherent to the slide, after which they were dried for several minutes in front of a fan. They were then immersed in solutions, one including and one lacking the appropriate substrate. Other sections were fixed for varying periods in 4 % formol saline or acetone, with resultant partial inhibition of enzyme action, but better localization of the diffusible products of the reaction. Nerve fibres were demonstrated by Bielschowsky's method for frozen sections and by Ranson's pyridine silver block impregnation. Unsuccessful attempts were made to demonstrate mitochondria by Altmann's technique.

For the phosphotungstic acid-Congo-red technique (Matsuura, 1925; modified by Swettenham & Sloper, in preparation), formol-fixed paraffin sections were stained for 30 min. in 1 % aqueous Congo red (B.D.H., 'biologically tested') to which a few drops of 0.75 % aqueous phosphotungstic acid had been added. They were then rinsed, and subsequently immersed in freshly made 0.75 % aqueous phosphotungstic acid for 1 min., blotted, and taken immediately to absolute alcohol and thence to xylol, and mounted in Canada balsam. The staining time in Congo red was reduced to 10 min. if sections were first reduced for 30 min. in sodium thioglycollate at pH 8.4.

Gomori's chrome-haematoxylin and aldehyde-fuchsin techniques were applied to tissues post-fixed in Bouin's fluid following Bargmann, Hild, Ortmann & Schiebler

(1950) and Dawson (1953) respectively. The first technique was also applied to sections post-fixed in Bouin's fluid with the addition of chrome-alum (4 g. per 100 ml.). Aldehyde fuchsin was at first only used when exactly 4 days old, but it was subsequently shown to retain its properties if kept at 4° C. for at least a month. 'Revector' basic fuchsin was suitable. It was found that adequate staining of N.S.M. with both techniques was obtained if sections were preoxidized for 5 min. in a mixture of equal parts of 0.3 %  $\text{KMnO}_4$  and  $\text{H}_2\text{SO}_4$ . This differs slightly from the concentrations recommended by Bargmann *et al.* (1950), namely a mixture of equal parts of 0.25 % permanganate and 0.5 % acid for 3 min.

Histochemical procedures, save where full references have been given, and save when the cryostat was employed, were as described in Pearse's *Histochemistry* (1953*a*). However, the periodic acid-Schiff reaction was performed in dog and cat following the technique recommended by Pearse (after Hotchkiss), with one modification, the use of a slightly different Schiff solution (Carleton & Leach, 1938). Sections from two dogs injected twice daily for 1 and 2 days respectively with 3-hydroxy-2-phenyl-cinchoninic acid (about 150 mg. per kg., following Bodian, 1951) were treated differently. These sections, which contained abundant N.S.M., were subjected to the above periodic acid-Schiff technique, save that diverse Schiff solutions were used. These Schiff solutions were made up and employed according to de Tomasi (1936), Barger & Delamater (1948), and Coleman (1938, as recommended by Lillie); they differed from each other slightly in the proportion of hydrochloric acid and sodium metabisulphite (or thionyl chloride) used. Immersion in these solutions was followed in each instance by the rinsing of one section, but not a second, in a reducing mixture before the sections were washed in tap water. The results obtained did not appreciably differ from each other. Further sections were treated as Schiebler (1952*b*) recommended, for which purpose a Schiff solution was made up according to Coleman (1938). Schiebler's technique differed from the others in that the solution of periodic acid was unbuffered; Hotchkiss's reducing rinse was omitted after oxidation of the section in periodic acid; immersion in Schiff's solution was prolonged to 1 hr.; and a rinse in a reducing solution followed rather than preceded the final washing of the section in tap water. His technique was also applied, modified in the following different ways: first, by the substitution of a buffered solution of periodic acid; secondly, by the insertion of Hotchkiss's reducing rinse; thirdly, by half an hour's immersion in Schiff solution; and fourthly, by a combination of the first and second modifications. From these variations it became clear that the differences between Schiebler's technique and our own depended very largely on the use by him of unbuffered periodic acid.

For the demonstration of *ascorbic acid* a cat was killed by the intracarotid injection of 5 % glucose in isotonic saline into the right carotid artery, followed by 5 % aqueous silver nitrate: the hypothalamus and pituitary were then dissected free and subjected to Bacchus's technique (1950). For the demonstration of *cystine*, paraffin sections were immersed (after treatment with wax solvents) in a fresh solution of sodium thioglycollate at pH 8.4: they were then washed in distilled water, and immersed in a mixture of freshly prepared 1 % aqueous solution of potassium ferricyanide (1 part) and ferric chloride (3 parts) for 1½ min. Subsequently they were washed in tap water, dehydrated and mounted (method of Hardy, 1952,



modified by Adams, 1954). Adjacent sections were similarly treated, save first, that the initial reduction with sodium thioglycollate was omitted; and secondly, that after this initial reduction, sections were immersed for 3 hr. in a 1 % aqueous solution of mercuric chloride: they were then washed in running tap water, and placed in the solution of ferric-ferricyanide.

## FINDINGS

### *Enzyme localization*

Unfixed sections required only a short incubation, in the case of phosphatases, of between 30 min. and 3 hr., and of esterases, of between 15 min. and 1 hr. Cytological details were not easily seen, although the shape of large neurones was discerned. If an enzyme was localized in nuclei alone, it could not be decided whether the nuclei were glial or neuronal. The enzymes studied were widely distributed, and their apparent concentration in certain nuclei may have reflected no more than the size and grouping of the neurones in these nuclei.

Thus acid phosphatase (Pl. 1, figs. 1, 2), splitting  $\alpha$ -glycerophosphate at pH 5 (Bourne, 1953) was conspicuous in the cytoplasm of many neurones in the hypothalamus. The neurones of the supraoptic and paraventricular nuclei were readily distinguished by their strong reaction. Numerous cells, possibly pituicytes, in the infundibular process gave a positive although less intense reaction. This technique was performed on unfixed sections, and on sections post-fixed in 4 % formalin for 1 hr. Sections were incubated for up to 3 hr. Negative results were obtained following the addition of 0.1 M sodium fluoride.

Alkaline phosphatases, splitting  $\alpha$ -naphthyl-phosphate (Pearse: incubation time up to 1 hr.) and  $\alpha$ -glycerophosphate (Gomori, 1952; incubation time 1–3 hr.) at pH 9.2 were conspicuous in the walls of vessels both in and round the infundibular process, and in the hypothalamus. Throughout the sagittal sections of hypothalamus and pituitary the nuclei of unidentified cells contained enzymes splitting adenosine-3-phosphate, adenosine triphosphate, and adenosine-5-phosphate (Pearse & Reis, 1952) at pH 7.5 (incubation time 2–3 hr.).  $\alpha$ -Naphthyl-phosphate proved an unsatisfactory substrate at pH 5 (Grogg and Pearse, 1952). Esterases were studied which attacked  $\alpha$ -naphthyl-acetate and  $\beta$ -naphthyl-acetate (Ravin, Sumner, Seligman, 1953). The reaction, whether on unfixed tissue, or tissue post-fixed in formalin or acetone for 1 hr. (when incubation was prolonged from 15 min. to 1 hr.), was very rapid and accompanied by diffusion. In many neurones, some of them in the position of the supraoptic and paraventricular nuclei, there was an intense cytoplasmic reaction. No comparable reaction was obtained from any structure in the infundibular process.

### *The staining of 'neurosecretory material'*

The chrome-haematoxylin, aldehyde-fuchsin, and phosphotungstic acid-Congo-red techniques clearly demonstrated material, respectively deep blue-black, purple and blue, in the cytoplasm of the neurones of the supraoptic and paraventricular nuclei, in beaded fibres passing from these neurones towards the pituitary, in similar fibres and larger aggregations in the infundibular stem, and in large and fine granules and fibres throughout the infundibular process (Pl. 1, figs. 3, 4). It is of interest that

the aldehyde-fuchsin and chrome-haematoxylin techniques involve an initial oxidation: conversely, the staining of N.S.M. with the phosphotungstic acid-Congo-red technique was potentiated by an initial reduction in sodium thioglycollate. The exact relationship of the N.S.M. to the neurones and their axones was not apparent. Much N.S.M. was clearly intracytoplasmic, while there were, especially in the dog, large aggregations in the region of the supraoptic nucleus which were not obviously lying in neurones: these, as Jewell (1953) has shown, were probably tangential sections of large neurones. In frozen sections from Bouin-fixed tissue derived from the cat the distribution of N.S.M. could be discerned, but there was a great increase in background staining.

In all three techniques elastic fibres in the walls of vessels and granules in the basophil cells of pars distalis of the pituitary were intensely stained. Certain other structures in the brain besides N.S.M. were also stained fairly deeply. Thus in the aldehyde-fuchsin technique, nerve fibres were lightly stained, while coarse cytoplasmic granules in many neurones, for example in the nuclei of the third cranial nerve (Pl. 1, fig. 5) were deeply stained. These differed from N.S.M. merely in their morphology and intensity of staining. Similar granules were demonstrated with sudan black and the periodic acid-Schiff technique; some also were slightly brown in unstained sections, some fluoresced in ultraviolet light, and some reduced ferric ferricyanide to a blue-green pigment without previous reduction of the tissue. These are all properties shared in varying degree by granules recognized in neurones, some, presumably lipofuscins, and others, so-called 'glyco-lipid' granules (Dixon & Herbertson, 1950).

The chrome-haematoxylin technique stained similar granules (recognized after immersion of the section in ribonuclease to remove Nissl substance), but more weakly than the aldehyde-fuchsin techniques. In contrast to the latter, it also stained nuclei and Nissl substance, the latter a pale blue-grey colour which seemed to differ from the staining of N.S.M. more in intensity and morphology than hue. The nature of intraneuronal substances other than N.S.M. stained by the phosphotungstic acid-Congo-red technique has not yet been studied: but it must be emphasized that this technique has quite as strong an affinity for N.S.M. as the other two (Pl. 1, fig. 6).

*General localization of lipids, carbohydrates and proteins in hypothalamus and neurohypophysis with particular reference to the distribution of 'neurosecretory material'*

*Lipids.* In unfixed frozen sections acetal-phospholipid (Hayes, 1949) was diffusely distributed throughout the infundibular process and peripheral zone of the infundibular stem. There was no other strong localization. Similar unfixed sections, post-fixed in weak Bouin's fluid or in formol calcium dichromate, showed a similar sudanophilia, abolished in the former by pyridine extraction. No strong localization of lipids in neurones was noted. Frozen sections from tissue fixed in formol dichromate, whether stained in sudan black or acid haematein, showed a similar diffuse but weaker reaction for lipid in the infundibular process and stem, but only a slight reaction in the cell bodies of some neurones, for example in the supraoptic nucleus. These studies were made on the cat. Paraffin sections from the dog and

cat, even after formol-calcium fixation and dichromation, failed to demonstrate any but the weakest sudanophilia in infundibular stem or process: but scanty material in the infundibular stem suggestive of Herring bodies showed slight sudanophilia, while cell bodies of many neurones of the supraoptic and paraventricular nuclei contained sudanophilic material. In one instance the sudan black was removed by 80 % alcohol and the section was then submitted to the aldehyde-fuchsin technique. Successive photographs revealed that only in some neurones had sudanophilic material the same distribution as N.S.M. (Pl. 2, fig. 7). (Photographs of sections treated successively with toluidine blue and the aldehyde-fuchsin technique showed a similar inconstant overlap between Nissl substance and N.S.M.) As for lipochrome, there was no pigmented material in the distribution of N.S.M. There were, however, fluorescent granules (in ultraviolet light at 3600 Ångströms) in neurones in the supraoptic nuclei of dog and cat, similar in distribution to those demonstrated with the periodic-acid Schiff technique: and there were many fluorescent granules in cells, probably pituicytes, in the infundibular process of a cat, but these were scanty in the infundibular process of the single dog examined in this way. This material lacked the distribution of N.S.M.

To summarize, the distribution of sudanophilic material differed in unfixed-frozen, formol-fixed-frozen, and paraffin-embedded sections. The first revealed abundant phospholipid in the infundibular process; the second, less lipid in that region, but some lipid in neurones of the supraoptic and paraventricular nuclei. In paraffin sections, in which N.S.M. is normally demonstrated, intraneuronal sudanophilic material was readily demonstrated, but had not the exact distribution of N.S.M. Apart from the faint sudanophilia of some Herring bodies, there was no strong sudanophilia in infundibular stem or process. Material fluorescing in ultraviolet light was widespread, but also lacked the distribution of N.S.M. This material was not identified.

*Carbohydrates.* Material, probably ascorbic acid, was demonstrated in occasional neurones of the supraoptic nucleus of the cat, but not in the infundibular stem or infundibular process. Methods for the demonstration of mucopolysaccharides (with 1 % aqueous Toluidine blue, and staining with Alcian blue (Steedman, 1951)) were also negative in the peripheral distribution of N.S.M. With the periodic acid-Schiff technique, which demonstrates unsaturated lipids as well as carbohydrate, material within the bodies of neurones of the supraoptic and paraventricular nuclei reacted, but there was no definite reaction in the infundibular stem or process (Pl. 2, fig. 8), save in occasional Herring bodies. With one modification of the periodic acid-Schiff technique (Schiebler, 1952*b*), Herring bodies were more evident, but neither beaded fibres nor material diffusely distributed throughout the infundibular process gave a definite reaction. Conversely, human collagen fibres, not demonstrated by our technique, reacted strongly. It was concluded that, irrespective of the results obtained with Schiebler's technique, no carbohydrate had the exact distribution of N.S.M.

*Protein.* With the Millon (Pollister, 1950) and Sakaguchi (Carver, Brown & Thomas, 1953) tests there was a faint but definite reaction in neurones of the supraoptic nuclei, in peripheral aggregations suggestive of Herring bodies, and diffusely throughout the infundibular process. But the colours developed in thin sections with these reactions were, at their best, poor.



*The nature of 'neurosecretory material'*

N.S.M. does not contain nucleic acids. Thus its staining properties were little affected by incubation of a section in a buffered solution (0.1 mg. per ml. at pH 8) of crystalline ribonuclease (Armour) for 2-3 hr., a period sufficient to remove the pyroninophilia of Nissl substance. Further, the Feulgen reaction for desoxyribose nucleic acid was negative at the sites of N.S.M. The staining properties of N.S.M. were also little affected by treating paraffin sections from formol-fixed tissue for 24 hr. in chloroform-methanol at 58° C. This strongly suggested that N.S.M. was not a lipid, but was inconclusive with regard to its central distribution, since sudanophilic material still persisted after extraction in some neurones of the supraoptic nucleus. Immersion of the fresh tissue in various fat solvents, for example 80 % alcohol or a mixture of chloroform (one part) and absolute alcohol (two parts) at room temperature for 24 hr., or, again, in chloroform and methyl alcohol in equal parts at 58° C. for a similar period, largely removed N.S.M., if the sections from the resultant paraffin-embedded tissue were floated on water. But the floating on Bouin's fluid of sections from the paraffin-embedded alcohol-extracted tissues, or the fixation of the alcohol-extracted tissues in formalin before paraffin embedding, preserved much N.S.M. in the infundibular process (Pl. 2, fig. 9), thus indicating that alcohol extraction did not remove all N.S.M. The second procedure was the more effective. The aldehyde-fuchsin technique no longer demonstrated beaded fibres or large peripheral aggregations of N.S.M. in the infundibular stem: but a weak reaction was present in some neurones of the supraoptic and paraventricular nuclei, while stainable material was abundant, although less so than in unextracted tissues, in the infundibular process. These observations can be explained by supposing that N.S.M. is a protein closely related in its central distribution to a lipid or lipo-protein. They accord well with the entire disappearance of N.S.M. after 3 hr. incubation of a section in crystalline trypsin (Armour, 0.1 mg. per ml.).

*Reactions for cystine*

Three methods were applied, all fundamentally demonstrating the presence of reducing substances. The first involved the reduction of dihydroxydinaphthylidissulphide by free sulphhydryl groups (Barnett & Seligman, 1952). These are present in tissues as such (in cysteine), but may also be derived from cystine by reduction. In tissue fixed in 1 % trichloroacetic acid in 80 % ethyl alcohol to preserve cysteine, although large neurones, for example in the supraoptic nucleus, contained material which reacted strongly, the reaction in the infundibular stem and process was weak and diffuse: a still weaker reaction was obtained in formol-fixed tissues, confirming that free reducing groups (e.g. —SH groups) did not share the exact distribution of N.S.M. However, the subsection of formalin-fixed tissues to reduction with 1 % sodium thioglycollate (Barnett & Seligman, 1954) was followed by a moderately strong positive reaction in the distribution of N.S.M. (Pl. 2, fig. 10), probably due to sulphhydryls derived from the reduction of the disulphide in cystine.

This reaction, however, demonstrated fairly intensely widespread background and intraneuronal material which was not apparent with two other methods. One of

these involved the production of formazans (Pl. 2, fig. 11). Sections were incubated at 54° C. in alkaline solutions of blue tetrazolium chloride and 2:5-diphenyl-3-4-styrylphenyl tetrazolium chloride (M. & B. 1767) (Pl. 2, fig. 11). The formazan produced by the former was sufficiently alcohol-insoluble to be mounted in balsam: its production is held by Pearse (1953*b*) to indicate the presence of cystine or cysteine in the absence of material reacting with the performic acid-Schiff and periodic acid-Schiff techniques. Since these latter reactions were largely negative in the peripheral distribution of N.S.M., i.e. in the infundibular stem and process save in some Herring bodies, it seemed probable that formazans produced in these areas at the site of N.S.M. were due to the presence of cystine or cysteine. However, Pearse's reaction was unsatisfactory save for observations made at low magnifications (e.g.  $\times 20$ ), since it was accompanied by a widespread granular precipitate: further, specific inhibitors such as mercuric chloride, could not be used.

Table 1. *Properties of neurosecretory material*

Method of demonstration in paraffin section	After trypsin (3 hr.)	After ribonuclease (3 hr.)	Alcohol extraction of fixed tissue	Alcohol extraction of unfixed tissue	After mercuric chloride (30 min.)
Chrome-haematoxylin	—	+	+	±*	.
Aldehyde-fuchsin	—	+	+	±*	.
Ferric-ferricyanide reduction (after reduction in sodium thioglycollate)	—	.	+	±*	—
Dihydroxydinaphthyl- disulphide reduction (after reduction in sodium thioglycollate)	—	.	+	±*	—
Alkaline tetrazolium	—	.	+	.	.

\* Limited reaction, depending on after-treatment.

An equally intense reaction without these disadvantages was obtained with Adams' ferric-ferricyanide reaction (Pl. 2, fig. 12). Material with the exact morphology and distribution of N.S.M. was clearly and immediately demonstrated a deep blue (Prussian blue). This reaction (Table 1) was negative after incubation of the section in trypsin (removing proteins), or the interposition of immersion in 1% mercuric chloride (blocking sulphhydryl groups, including those revealed by sodium thioglycollate): and it was positive after the extraction of the paraffin sections in a mixture of chloroform and methanol at 58° C. In sections untreated with sodium thioglycollate occasional intraneuronal granules, probably containing lipofuscin, reduced ferric-ferricyanide promptly to give a blue-green pigment. Such granules were not seen in the distribution of N.S.M. in the dogs and cats studied. The selective way in which N.S.M. is demonstrated by Adams' technique is probably a reflection on the relative insensitivity of this technique, and its inability to demonstrate small amounts of cystine.

#### DISCUSSION

The extraction of substances with oxytocic, vasopressor and antidiuretic activity from the hypothalamus and infundibular process is compatible with their manufacture in either region or in both. Three methods have been applied here to the

study of this problem in the dog and cat, the first concerned with the relative distribution of enzymes in the two regions, the second related to the tinctorial specificity, and the third to the histochemistry, of Bargmann's 'neurosecretory material'.

### *Enzyme localization*

Attempts at enzyme localization suffered from four technical shortcomings: first, at least 5 min. elapsed between death and the freezing of tissues to  $-70^{\circ}\text{C}.$ ; secondly, this freezing, although rapid, was unaccompanied by dehydration; thirdly, sections had to be thawed and briefly dried before immersion in substrate; and fourthly, good localization postulates limited diffusion of enzyme, substrate and reaction product: a result best achieved by fixation which may inhibit the enzyme.

However, in the cat the cytoplasm of neurones of the supraoptic and paraventricular nuclei was rich in enzymes splitting sodium  $\alpha$ -glycerophosphate at pH 5, and  $\alpha$ -naphthyl acetate. Enzymes splitting adenosine-5-phosphate, adenosine-3-phosphate and adenosine triphosphate (ATP) at pH 7.5 showed a generalized distribution in the nuclei of unidentified cells throughout the hypothalamus and pituitary: alkaline  $\alpha$ -naphthyl phosphatase and  $\alpha$ -glycerophosphatase were conspicuous in the walls of vessels in both regions.

The only comparable observations on the cat are those of Schiebler (1951), who, confirming Wislocki & Dempsey's (1948) observations on the macaque, recorded a marked reaction for acid and alkaline phosphatase in the cytoplasm of the supraoptic neurones in paraffin-embedded tissue. The incubation times employed by these workers were very long, and render their findings somewhat dubious: certainly in the cat we failed to obtain a cytoplasmic reaction for alkaline phosphatase. However, Eränkö's (1951) localization of acid phosphatase in the 'magnocellular' hypothalamic neurones of the rat in frozen formalin-fixed tissues, using a short incubation time, is entirely acceptable. The distribution of esterases was widespread, and conforms with the general distribution in the hypothalamus noted for these enzymes by Gomori & Chessick (1953). A similar reaction for esterases was lacking in the infundibular process, but in numerous cells, probably pituicytes, there was a cytoplasmic reaction for acid phosphatase, a reaction less conspicuous there than in the hypothalamus. No comparable observations on the infundibular process have hitherto been made, although in man Pearse & Reis (1952) noted a diffuse extracellular localization of adenosine-5-phosphatase, which we could not confirm in the cat.

Acid phosphatase and esterase were the only two enzymes which gave a conspicuous cytoplasmic reaction in neurones of the supraoptic and paraventricular nuclei. These reactions were of such intensity as to suggest that their study might be some indication of neuronal activity, for example in animals deprived of water. The significance of the weak or negative reactions obtained for these enzymes in the infundibular process depends upon the extent to which they may be associated with the secretion of posterior-lobe hormone.

This association is highly speculative. In so far as acid-phosphatase may be related to protein synthesis, its strong localization in the supraoptic and paraventricular nuclei somewhat favours the secretion of hormone in the hypothalamus.



With regard to esterase, evidence has been adduced concerning its identification, at least in part, with acetylcholine esterase (Ravin *et al.*, 1953). This enzyme is probably closely concerned with the liberation of antidiuretic hormone (Pickford, 1938; Harris, 1948), and, while its localization in the supraoptic neurones is compatible alike with hormone synthesis in hypothalamus and pituitary, its demonstration in the pituitary would have strengthened the view that the posterior lobe hormones were formed there. It must, however, be emphasized that our attempts at enzyme localization were at best crude; positive results were of some value, but negative results must be regarded with the greatest reserve.

#### *The staining of 'neurosecretory material'*

Gomori's chrome-haematoxylin technique was the first stain unequivocally to demonstrate N.S.M. in the hypothalamus and neurohypophysis in mammals. Schiebler (1951) subsequently established that after appropriate preoxidation a variety of nuclear stains behaved similarly. The next step lay in the staining of N.S.M. with Gomori's aldehyde-fuchsin technique (Dawson, 1953). This stain is particularly satisfactory. It has no affinity for nuclei, but like the chrome-haematoxylin and phosphotungstic acid-Congo-red techniques, it has an affinity for elastic and pituitary basophile cells.

Of these staining methods that using chrome-haematoxylin has been the most studied, and there seems a certain tacit or express (Smith, 1951) acceptance of its specificity. It is undeniable that in sections of the neurohypophysis, hypothalamus, and neighbouring brain there are no comparable aggregations of stainable material. On the other hand, distinctions such as that made by Bargmann (1949) between the violet staining of Nissl substance and deep blue of N.S.M. must be accepted with reserve: for such distinctions, to our mind, depend more on intensity of staining and morphology than hue. The same is true of the specificity of the aldehyde-fuchsin technique, which reveals coarse granules in many neurones, for example in those of the third cranial nerve nucleus. Similar granules are also stained, but less intensely, by the chrome-haematoxylin technique. The exact identity of these granules has not been determined, and they are not necessarily homogeneous, for some few were light brown in unstained sections, whereas most were colourless. The histochemical reactions of similar granules were those of lipofuscins or of the 'glycolipid' granules of Dixon & Herbertson (1950). It is important to note that these histochemical properties were not shared by any material with the exact distribution of N.S.M. In short, the staining techniques used were of limited specificity, unless account was taken of intensity of staining and morphology. The fact that three entirely different techniques had a common affinity for N.S.M. greatly favoured the homogeneity of this stainable material throughout its distribution. The chemical basis for this common affinity was not determined.

#### *Histochemical observations on the neurohypophysis*

A variety of substances share the distribution of N.S.M. Schiebler (1952*a, b*) particularly stressed the presence of protein (Millon positive), carbohydrate (periodic acid-Schiff positive even after acetylation) and sudanophilic lipid. The last two

differed somewhat in their distribution from N.S.M., but were still held to be part of it. This variable distribution he established for lipid by the successive staining of the same section with sudan black and chrome-haematoxylin. This was apparently done on frozen sections, which in our hands are unsatisfactory since they involve a loss in the specificity of the stains for N.S.M. Indeed, we feel that none of these techniques adequately demonstrates aggregations of lipid or carbohydrate in the exact distribution of N.S.M. Thus carbohydrates, in so far as they are demonstrated by the periodic acid-Schiff technique, were conspicuous in neurones of the supraoptic nucleus. A weak reaction was also given by material in the infundibular stem and process, probably Herring bodies. These bodies were much more in evidence if the periodic acid was used in unbuffered solution, as seems to have been the case in the method adopted by Schiebler (1952*b*). This modification is regarded with disfavour by Pearse (1953*a*), and indeed in our hands Schiebler's technique allows the ready demonstration of protein structures such as collagen. However, whatever the validity of the reaction as Schiebler performed it, in our hands the bulk of N.S.M. in the infundibular process reacted at best only very weakly. Similarly, other techniques for demonstrating carbohydrates, for example acid mucopolysaccharide and ascorbic acid, were not positive throughout the distribution of N.S.M. This applied also to lipids, as demonstrated, for example, by their sudanophilia in paraffin sections. As for proteins, the two methods commonly employed, the Sakaguchi and Millon reactions for arginine and tyrosine, proved in our hands, at their best, unsatisfactory; although a faintly positive reaction was given in the cat by material with the distribution of N.S.M. It was concluded at this stage that, if N.S.M. was a single homogeneous substance it was, by exclusion, probably a protein.

#### *The properties of 'neurosecretory material'*

The conditions necessary for the staining of N.S.M. were next analysed. These were at their best in sections from paraffin-wax-embedded formol-fixed tissues, conditions more suggestive of a protein than lipid material. This was corroborated by the entire removal of N.S.M. by trypsin, and by its persistence in paraffin sections after prolonged immersion in hot chloroform-methanol.

However, according to Schiebler (1951) and Hild & Zetler (1952-3) N.S.M. is entirely removed from the unfixed neurohypophysis by alcohol extraction, although the hormonal activity persists in the alcohol-extracted tissue. Conversely, this activity is lacking from the alcoholic extract, although the latter contains chrome-haematoxyphil material. It is therefore suggested, first that there is a major lipid component in N.S.M.: and secondly that N.S.M. represents a bearer-substance, and not the hormone itself. However, mindful of the great loss of water-soluble material which can occur when paraffin sections are floated on water in course of preparation (J. Vallance-Owen, 1950), we floated paraffin sections from alcohol-extracted otherwise unfixed tissues on Bouin's fluid. We then obtained a strong positive reaction for N.S.M. in the infundibular process. N.S.M. was better preserved if alcohol-extracted tissues were transferred to formalin prior to embedding. This is not to deny that alcohol-extraction does remove stainable material, in particular from the neurones of the supra-optic and paraventricular nuclei, where it is closely associated with a

lipid and present in less quantity than in the infundibular process. But it is clear that a large part of N.S.M. is not alcohol-soluble, and like posterior-lobe hormone, persists in the neurohypophysis after alcohol extraction.

The last step lay in finding a strong positive histochemical reaction for N.S.M. In view of the affinity of the aldehyde-fuchsin and chrome-haematoxylin techniques for the supposedly insulin-rich pancreatic islet  $\beta$  cells, and because of the high content of cystine in insulin, an attempt was made to localize cystine in the neurohypophysis. Such reactions depend on the reducing properties of sulphhydryl ( $-SH$ ) groups freed by the reduction of cystine. Three methods were applied. The first, involving the reduction of dihydroxydinaphthylsulphide has already been claimed by Barnett & Seligman (1954) to demonstrate posterior-lobe hormone in the infundibular process of the rat. However, this reaction, perhaps because of its sensitivity, does not distinguish N.S.M. as clearly as the staining techniques commonly employed: for although the material can be recognized, adjacent tissues give a fairly marked reaction. A more selective reaction is that with alkaline tetrazolium, which Pearse (1953*b*) noted, but without interpretation, to be positive in the human infundibular process. The strongly positive result obtained in the infundibular process of cat and dog may be regarded as indicative of cystine in those areas where lipid and carbohydrate can be excluded. These reservations limit the value of this reaction. Further, it is unsatisfactory for detailed microscopy, and compares unfavourably with a third, which involves the reduction of ferric ferricyanide to Prussian blue by substances only revealed by the prior reduction of the tissue by sodium thioglycollate.

#### *The interpretation of the ferricyanide reaction*

Adams' modification of the ferricyanide reaction is rigorous in that it postulates the immediate development of Prussian blue (as opposed to Prussian green) after brief immersion of the tissue in a mixture of potassium ferricyanide and ferric chloride. It is clearly an index of the presence of strong reducing groups. These groups were not present as such in sufficient amount to reduce ferric ferricyanide in the distribution of N.S.M. in dog and cat, but were revealed when the tissue had been reduced by sodium thioglycollate. Since these groups were blocked by mercuric chloride, they were probably sulphhydryl ( $-SH$ ) groups, presumably derived by reduction from the disulphide ( $S-S$ ) in cystine. The fact that trypsin prevented the reaction from occurring corroborated the protein source of these reducing groups. But it cannot be taken that the specificity of either mercuric chloride or trypsin is absolute: nor, again, can it be assumed that there are not other unidentified proteins in tissues with similar properties.

Further, although it is probable that the reaction is primarily due to a protein, the potential contribution of lipid- or carbohydrate-reducing substances in the neurones of the supraoptic and paraventricular nuclei, where these are rich, is difficult entirely to exclude. These qualifications aside, it is most likely that we are concerned with a protein, fixed *in situ* by formalin, and containing an unusual concentration of cystine.

This material has a close similarity with the oxytocic and vasopressor cyclic octapeptides of du Vigneaud and his colleagues, in that these octapeptides contain



a high proportion of cystine. By inference, therefore, it is possible that N.S.M. represents, at least in part, posterior-lobe hormone. This inference will have to be confirmed by physiological experiments similar to those by which the significance of N.S.M. has been established.

### CONCLUSIONS

Our observations corroborate Bargmann's theory of neurosecretion, and indicate a variety of techniques applicable to the physiological and pathological investigation of the neurohypophysis. Thus, with regard to the relative distribution of enzymes in this region, it is likely that the study of esterases and of acid phosphatases will be rewarding.

With regard to the specificity of the stains used for the demonstration of N.S.M., this is limited: for Nissl substance, and granules probably akin to lipofuscin or 'glycolipid' inclusions, are also stained in varying degree by the chrome-haematoxylin and aldehyde-fuchsin techniques. On the other hand, it has been confirmed that no material in the hypothalamus or in its immediate vicinity has the morphology and intensity of staining of N.S.M. Further evidence in favour of the homogeneity of N.S.M. was obtained by the development of a third staining method, the phosphotungstic acid-Congo red technique, for its demonstration.

Since the chemical basis for these techniques is obscure, diverse histochemical tests were applied to discover the nature of N.S.M. It became clear that there was no great concentration of lipid or carbohydrate throughout the distribution of this material, an observation which accorded well with its loss after incubation in trypsin, and its persistence in paraffin sections after the alcoholic extraction of lipids. The alcoholic extraction of fresh unfixed tissues removed a certain amount of stainable material, but N.S.M. could still be demonstrated in large amounts in the infundibular process. In short, contrary to general opinion, N.S.M. lacked the properties of a carbohydrate or lipid.

It remained to find a histochemical technique for the identification of N.S.M., and this was achieved with a method which probably demonstrates high concentrations of cystine. Since certain recently described substances with high oxytocic and anti-diuretic activity are also rich in cystine, it is possible that the material demonstrated in sections was the hormone itself.

### SUMMARY

1. Current views on the formation of oxytocin, vasopressin and antidiuretic hormone are discussed.

2. In the cat the relative distribution of esterase and acid phosphatase in the hypothalamus as opposed to the infundibular lobe of the pituitary is compatible with hypothalamic neurosecretion.

3. In the dog and cat the chrome-haematoxylin and aldehyde-fuchsin techniques are shown to be of limited specificity when used for the demonstration of 'neurosecretory' material (N.S.M.) in the hypothalamus and neurohypophysis.

4. A different staining method, the phosphotungstic acid-Congo red technique, also demonstrates N.S.M.; the common affinity of these three different techniques for N.S.M. corroborates the view that N.S.M. is a single substance.

5. Histochemical evidence indicates that N.S.M. is not a glycolipoprotein, but is a protein rich in cystine. It is suggested that this material may represent certain cystine-rich octapeptides which show marked oxytocic vasopressor and antidiuretic activity.

I am particularly grateful to Mr Kenneth Swettenham for his technical assistance. Thanks are also due to Prof. Dorothy Russell, Prof. R. J. Harrison and Dr Bourne for help in the preparation of this paper; to Messrs May and Baker for the gift of tetrazolium salts; to Messrs Hopkins and Williams for the synthesis of dihydroxydinaphthylsulphide and to Mr A. L. Gallup for the photography.

#### REFERENCES

- ADAMS, C. W. M. Personal communication.
- BARGMANN, W. (1949). Über die neurosekretorische Verknüpfung von Hypothalamus und Neurohypophyse. *Z. Zellforsch.* **34**, 610–634.
- BARGMANN, W., HILD, W., ORTMANN, R. & SCHIEBLER, T. H. (1950). Morphologische und experimentelle Untersuchungen über das hypothalamisch-hypophysäre System. *Acta Neurovegetativa*, **1**, 233–275.
- BARNETT, R. J. & SELIGMAN, A. M. (1952). Histochemical demonstration of protein-bound sulphhydryl groups. *Science*, **116**, 323–327.
- BARNETT, R. J. & SELIGMAN, A. M. (1954). Histochemical demonstration of sulphhydryl and disulfide groups of protein. *J. nat. Cancer Inst.* **14**, 769–803.
- BODIAN, D. (1951). Nerve endings, neurosecretory substance, and lobular organization. *Johns Hopk. Hosp. Bull.* **89**, 355–376.
- BOURNE, G. H. (1953). *An Introduction to Functional Histology*, p. 173. London.
- CARLETON, H. M. & LEACH, E. H. (1938). *Histological Technique*, 2nd ed., p. 163. Oxford.
- CARVER, M. J., BROWN, F. C. & THOMAS, L. E. (1953). An arginine histochemical method using Sakaguchi's new reagent. *Stain Tech.* **28**, 88–91.
- COLEMAN (1938). Quoted in Gomori, G. (1952). *Microscopic Histochemistry*, p. 59. Chicago.
- COONS, A. H. & KAPLAN, M. H. (1950). Localization of antigen in tissue-cells. *J. exp. Med.* **91**, 1–29.
- DAWSON, A. B. (1953). Evidence for the termination of neurosecretory fibres within the pars intermedia of the hypophysis of the frog (*Rana pipiens*). *Anat. Rec.* **115**, 63–69.
- DIXON, K. C. & HERBERTSON, B. M. (1950). Clusters of granules in human neurones. *J. Path. Bact.* **62**, 335–339.
- DU VIGNEAUD, V., LAWLER, H. C. & POPENHOE, E. A. (1953). Enzymatic cleavage of glycineamide from vasopressin and a proposed structure for this pressor-antidiuretic hormone of the posterior pituitary. *J. Amer. chem. Soc.* **75**, 4880–4881.
- DU VIGNEAUD, V., RESSLER, C., SWAN, J. M., ROBERTS, C. W., KATSOYANNIS, P. G. & GORDON, J. (1953). The synthesis of an octapeptide amide with the hormonal activity of oxytocin. *J. Amer. chem. Soc.* **75**, 4879–4880.
- ERÄNKO, O. (1951). Histochemical evidence of intensive phosphatase activity in the hypothalamic magnocellular nuclei of the rat. *Acta physiol. scand.* **24**, 1–6.
- GERSH, I. (1939). The structure and function of the parenchymatous glandular cells in the neurohypophysis of the rat. *Amer. J. Anat.* **64**, 407–443.
- GOMORI, G. (1939). A differential stain for cell types in the pancreatic islets. *Amer. J. Path.* **15**, 497–499.
- GOMORI, G. (1952). *Microscopic Histochemistry*, p. 184. Chicago.
- GOMORI, G. & CHESSICK, B. D. (1953). Esterases and phosphatases of the brain; a histochemical study. *J. Neuropath.* **12**, 387–396.
- HARDY, M. H. (1952). The histochemistry of hair-follicles in the mouse. *Amer. J. Anat.* **90**, 285–329.
- HARRIS, G. W. (1948). Neural control of the pituitary gland. *Physiol. Rev.* **28**, 139–179.
- HAYES, E. R. (1949). A rigorous redefinition of the plasmal reaction. *Stain Tech.* **24**, 19–23.

- HILD, W. (1951). Experimentell-morphologische Untersuchungen über das Verhalten der 'Neurosekretorischen Bahn' nach Hypophysenstieldurchtrennungen, Eingriffen in den Wasseraushalt und Belastung der Osmoregulation. *Virchows Arch.* **319**, 526-546.
- HILD, W. & ZETLER, G. (1951). Über das Vorkommen der Hypophysenhinterlappenhormone im Zwischenhirn. *Arch. exp. Path. Pharmacol.* **213**, 139-153.
- HILD, W. & ZETLER, G. (1952-3). Über die Funktion des Neurosekrets im Zwischenhirn-Hypophysensystem als Trägersubstanz für Vasopressin, Adiuretin und Oxytocin. *Z. ges. exp. Med.* **120**, 236-243.
- JEWELL, P. A. (1953). The occurrence of vesiculated neurones in the hypothalamus of the dog. *J. Physiol.* **121**, 167-181.
- LAQUEUR, C. (1954). *Recent Progress in Hormone Research*, **10**, p. 233, edited by Pincus, G. New York.
- MATSUURA, S. (1925). Eine neue Färbungsmethode der elastischen Elemente, welche auch andere Fasern und Zellen in verschiedenen Nuancen abhebt. *Fol. anat. jap.* **3**, 107-110.
- PEARSE, A. G. E. (1953*a*). *Histochemistry*. London.
- PEARSE, A. G. E. (1953*b*). The histochemical demonstration of cystine-containing structures by methods involving alkaline hydrolysis (the alkaline tetrazolium method). *J. histochem. cytochem.* **1**, 460-468.
- PEARSE, A. G. E. & REIS, J. L. (1952). The histochemical demonstration of a specific phosphatase (5 nucleotidase). *Biochem. J.* **50**, 534-536.
- PICKFORD, M. (1938). The inhibitory effect of acetyl choline on water diuresis in the dog and its pituitary transmission. *J. Physiol.* **95**, 226-238.
- POLLISTER, A. W. (1950). As quoted by Gomori, G. (1952). *Microscopic Histochemistry*, p. 113. Chicago.
- RAVIN, H. A., SUMNER, I. Z. & SELIGMAN, A. M. (1953). The histochemical localization of acetylcholine esterase in nervous tissue. *J. Pharmacol.* **107**, 37-53.
- SCHARRER, E. & SCHARRER, B. (1937). Über Drüsen-Nervenzellen und neurosekretorische Organe bei Wirbeltiere und Wirbellosen. *Biol. Rev.* **12**, 185-216.
- SCHARRER, E. & SCHARRER, B. (1954). *Neurosekretion. Handbuch der mikroskopischen Anatomie des Menschen*, **6**, part 5, 953-1066. Edited by Möllendorf, W. Berlin.
- SCHIEBLER, T. H. (1951). Zur Histochemie des neurosekretorischen hypothalamisch-neurohypophysären Systems. *Acta Anat.* **13**, 233-255.
- SCHIEBLER, T. H. (1952*a*). Die chemischen Eigenschaften der neurosekretorischen Substanz in Hypothalamus und Neurohypophyse. *Exp. Cell. Res.* **3**, 249-250.
- SCHIEBLER, T. H. (1952*b*). Zur Histochemie des neurosekretorischen Hypothalamisch-Neurohypophysären Systems (II Teil). *Acta Anat.* **15**, 393-416.
- SMITH, S. W. (1951). The correspondence between hypothalamic neurosecretory material and neurohypophyseal material in vertebrates. *Amer. J. Anat.* **89**, 195-232.
- SWETTENHAM, K. & SLOPER, J. C. In preparation.
- VALLANCE-OWEN, J. (1950). The histochemical demonstration of glycogen in necropsy material. *J. Path. Bact.* **60**, 325-327.
- VOGT, M. (1953). Vasopressor, antidiuretic, and oxytocic activities of extracts of the dog's hypothalamus. *Brit. J. Pharmacol.* **8**, 193-200.
- WISLOCKI, G. B. & DEMPSEY, G. W. (1948). The chemical histology and cytology of the pineal body and neurohypophysis. *Endocrinology*, **42**, 56-72.
- ZUCKERMAN, S. (1954). The secretions of the brain. Relation of hypothalamus to pituitary gland. *Lancet*, **1**, 789-795.

## EXPLANATION OF PLATES

## PLATE 1

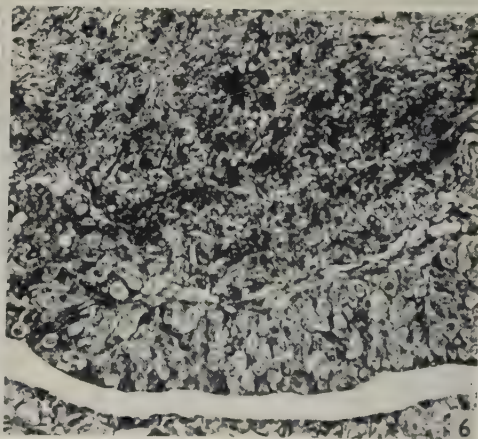
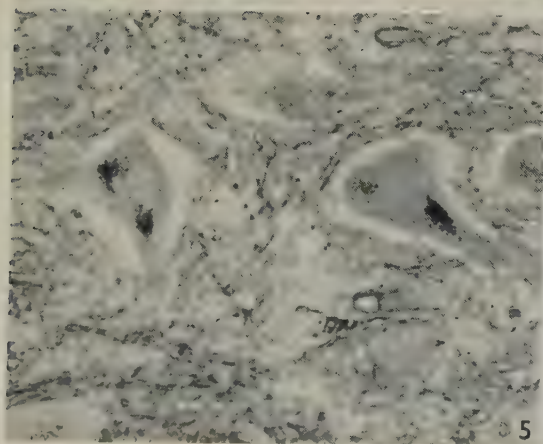
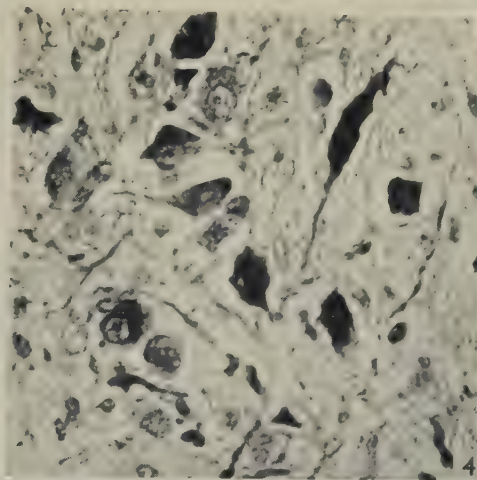
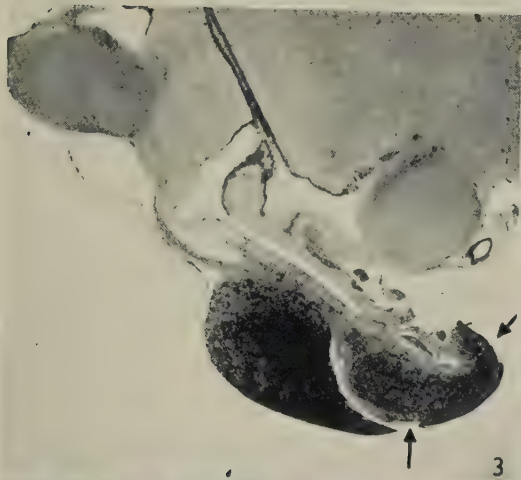
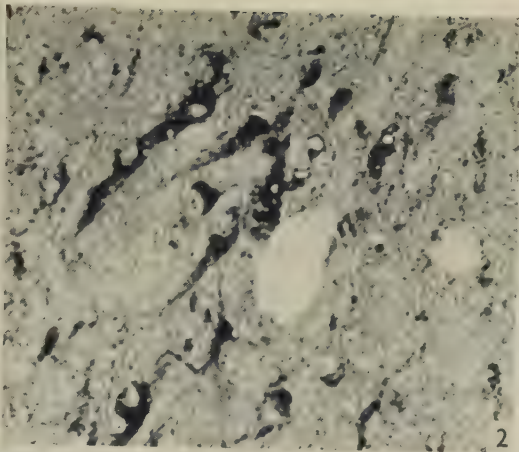
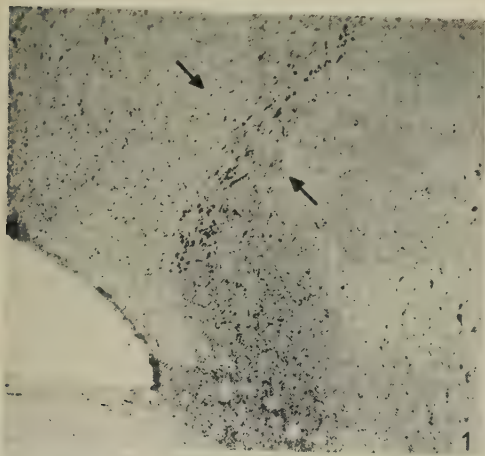
- Fig. 1. Sagittal section of hypothalamus of cat to show acid phosphatase in neurones of the paraventricular nucleus (arrows). Unfixed frozen section at  $7\mu$ . Sodium glycerophosphate at pH 5 as substrate,  $\times 26$ .
- Fig. 2. Hypothalamus of cat. To show cytoplasmic distribution of acid-phosphatase in neurones of the paraventricular nucleus,  $\times 275$ .
- Fig. 3. Sagittal section through cat neurohypophysis. Note deep staining of N.S.M. throughout infundibular process (arrows). Chrome-haematoxylin technique,  $\times 10$ .



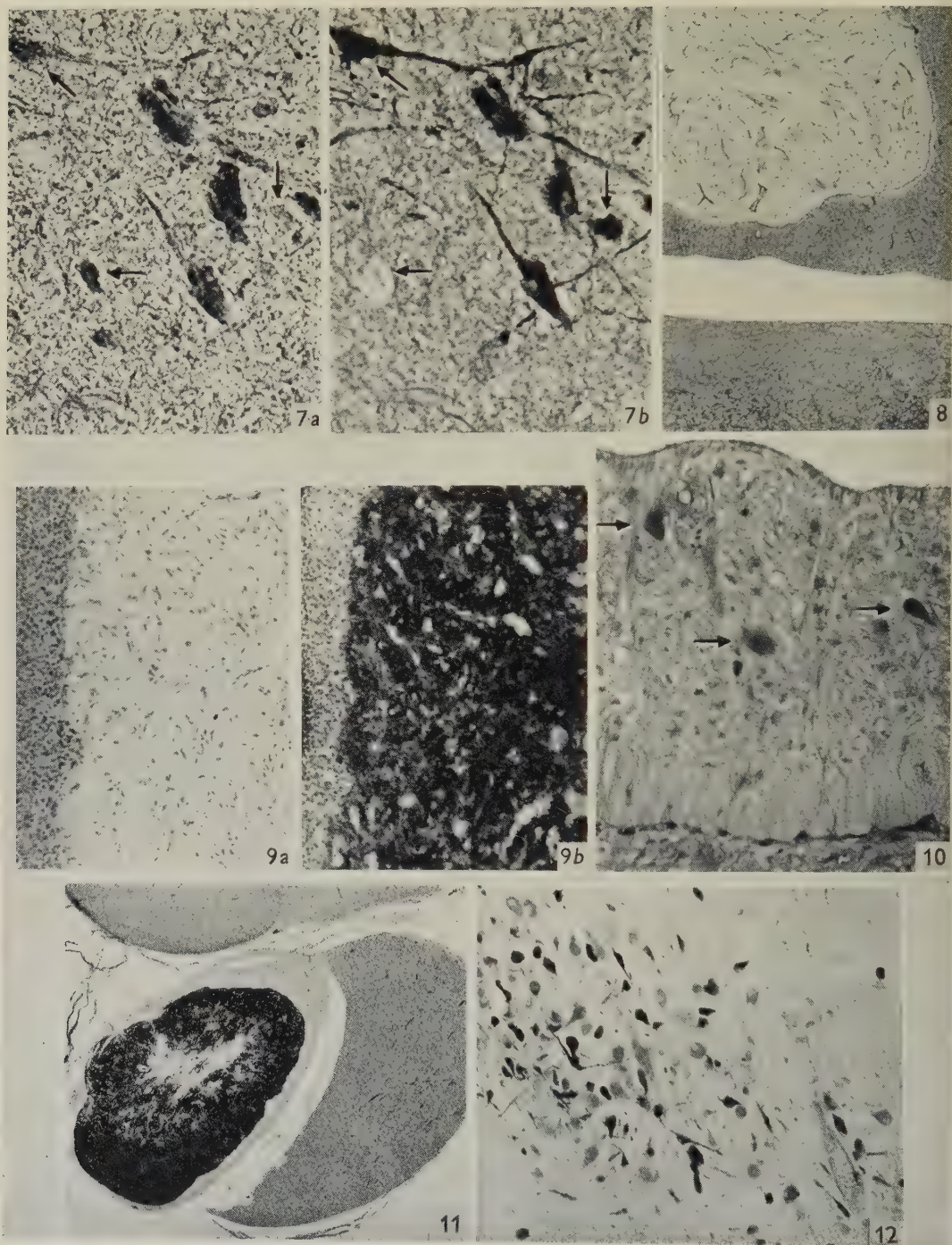
- Fig. 4. Supraoptic nucleus of cat. Note intraneuronal and axonal distribution of N.S.M., especially in beaded fibres. Chrome-haematoxylin technique,  $\times 365$ .
- Fig. 5. Neurones of the third cranial nerve nucleus of dog. To show intraneuronal granules staining similarly to N.S.M. Aldehyde-fuchsin technique,  $\times 350$ .
- Fig. 6. Infundibular process, pars intermedia, and pars distalis of dog. N.S.M. in infundibular process, both diffusely distributed and in aggregates. Phosphotungstic acid-Congo red technique,  $\times 145$ .

## PLATE 2

- Fig. 7. Paraventricular nucleus of cat. Note partly dissimilar distribution (arrows) of lipid (A) and N.S.M. (B) in section successively stained with sudan-black and aldehyde-fuchsin technique,  $\times 386$ .
- Fig. 8. Infundibular process of cat. To show negative periodic acid-Schiff reaction,  $\times 36$ .
- Fig. 9. Infundibular process of cat. Fresh tissue extracted in chloroform-ethyl alcohol mixture for 24 hr. Adjacent sections from resultant paraffin-embedded tissue, floated (A) on water (B) on Bouin's fluid, and stained on same slide, to show preservation of N.S.M. in (B). Chrome-haematoxylin technique,  $\times 134$ .
- Fig. 10. Infundibular stem of cat. To show large aggregates of reducing material (arrows), probably cystine, in inner zone of stem (abutting on ventricle). The distribution of N.S.M. is not shown as clearly as with the staining techniques. Reaction with dihydroxydinaphthyl-disulphide after reduction with sodium thioglycollate,  $\times 24$ .
- Fig. 11. Infundibular process of dog. To show reducing material (black), probably cystine. The distribution of N.S.M. can only be studied under low-magnification with this technique. Alkaline tetrazolium reaction (using M. & B. 1767),  $\times 17$ .
- Fig. 12. Paraventricular nucleus of dog, to show reducing material, probably cystine, in typical distribution of N.S.M. Reduction of ferric ferriocyanide after reduction in sodium thioglycollate,  $\times 75$ .









## STUDIES ON THE INNERVATION OF SKIN

III. THE PATTERNED ARRANGEMENT OF THE SPINAL  
SENSORY NERVES TO THE RABBIT EAR

BY G. WEDDELL, D. A. TAYLOR AND C. M. WILLIAMS

*Department of Anatomy, University of Oxford*

## INTRODUCTION

Although the average number of myelinated dorsal root nerve fibres entering the rabbit ear is now known, as well as the number of hairs (and hair follicles) with which it is endowed (see papers I and II), only three things seem to be known with any degree of certainty about the way in which the nerves are distributed in relation to the hairs. In 1941 Weddell showed that the nerves supplying the hair follicles, after leaving the main nerve trunks which lie immediately beneath the skin, break up into fasciculi which, by inosculation with one another, form a reticulate pattern corresponding closely to that formed by the blood vessels. Bundles of nerve fibres leave the fasciculi at intervals and enter the dermis where, together with numerous other nerve fibres, they form the so-called cutaneous nerve plexus. In this position they pursue a complicated course, dividing and subdividing a number of times as they approach closer to the surface of the skin to end in company with other nerve fibres in the layers of a hair follicle. In this way, the terminal branches of a single axon become scattered so that each and every group of hair follicles in the ear is supplied by nerve fibres which radiate from it in all directions. As the result of this arrangement, some nerve fibres actually pursue a recurrent course in the ear before reaching their destination.

Weddell also demonstrated that when about 25 % of the fibres of the great auricular nerve are severed proximal to the point at which it enters the ear, this does not result in the absence of nociceptive responses to pin-prick anywhere in the dorsum of the ear (and, presumably, to impulses from stimulated hairs) but that a number of degenerating nerve fibres intermingled with normal fibres can be seen throughout the skin of the back of the ear. The number of degenerating fibres, however, is greatest in that part of the ear supplied chiefly by the great auricular nerve. The collaterals of one degenerating fibre were found to extend over about 1 cm.<sup>2</sup> of skin in which there were approximately 2000 hairs. On the basis of this observation, it was concluded that a single nerve fibre might well supply a very large number of hairs. However, the practical difficulty of tracing all the collaterals associated with a single normal, let alone a degenerating axon over long distances without confusing them at any point with normal or degenerating collaterals derived from another parent axon is so great that an accurate estimation of the number of hair follicles supplied by a single nerve fibre by this means is not a practical proposition.

Finally, in the same series of degeneration experiments, Weddell demonstrated that many hairs in the rabbit ear are supplied by more than one nerve fibre, but

found it impossible to determine how many nerves might go to a single hair, or indeed whether each individual stem axon supplying a hair was related to the same or separate dorsal root nerve fibres.

Clearly, his degeneration experiments were of qualitative rather than quantitative value, so that in the first section of this paper additional observations resulting from the examination of normal material stained by improved histological techniques, as well as the results of further and more critical degeneration experiments, will be reported. However, as will be seen, these still failed to give us all the information which we wanted, owing to the limitations imposed by the purely morphological approach. To obtain further information, therefore, we carried out a number of experiments using the evoked action-potential technique.

The first part of this paper is concerned with an analysis of the arrangement of the fasciculi in the great auricular nerve and the way in which both the fasciculi and individual nerve fibres scatter in the ear. The observations are based upon: (1) the examination of unoperated preparations of skin from the dorsum of the whole ear either treated with osmium tetroxide or stained with methylene blue following the injection of hyaluronidase (Weddell & Pallie, 1954); and (2) the results of degeneration experiments (in which single small fasciculi from the great auricular nerve were divided (*a*) close to the vertebral column, (*b*) at the base of the ear) as seen in skin from the whole of the back of the ear, similarly stained with methylene blue following treatment with hyaluronidase.

The second part of the paper is concerned with: (1) the position, as well as the nature and density of innervation of the hairs in areas of ear skin subserved by single fasciculi (*a*) at the base of the ear, (*b*) close to the vertebral column from the named ear nerves as judged by the action potentials evoked when the hair stumps were stimulated: some of the fasciculi were excised and their nerve fibre content determined subsequently; (2) the areas of skin on the dorsum of the ear subserved by dorsal spinal nerve rootlets of the second and third cervical nerves between the dorsal root ganglia and the spinal cord when outlined in a similar manner.

#### MATERIAL AND METHODS

Rabbits not more than 6 months old of both Dutch and Copenhagen strains were used.

##### (1) *Unoperated material*

Skin from the whole of the back of the ear was stained in (*a*) osmium tetroxide (ten animals), (*b*) methylene blue (40 animals) following treatment with hyaluronidase and prepared for examination as a whole cleared specimen.

##### (2) *Degeneration experiments*

These were carried out in twenty-six animals as follows: the rabbit was anaesthetized and the great auricular nerve exposed at the selected position. A small fasciculus was then separated with the greatest care from its neighbours and excised with a sharp pair of scissors. For control purposes, the great auricular nerve on the opposite side was exposed at the same site and a small fasciculus similarly separated

from its neighbours, but not excised. Two of the animals in which the fasciculi had been excised close to the vertebral column were allowed to survive for 2 weeks. They were then killed and the great auricular nerves removed from both sides, placed in fixative and prepared for microscopical examination after staining by the Marchi method. The animals in which fasciculi had been excised at the base of the ear and three in which the fasciculi had been excised close to the vertebral column were allowed to survive for 48 hr. They were then anaesthetized and the skin throughout the back of both ears stained with methylene blue and prepared for microscopical examination as whole preparations. In three animals in which it was known (from electro-physiological observations, see below) that the fasciculus excised subserved hairs on the front as well as the back of the ear, relevant pieces of skin from the front of the ear were similarly prepared for microscopical examination.

### (3) *Electrophysiological technique*

To enable us to stimulate a single hair in isolation, as well as to evoke action potentials by brushing the hairs collectively, it was found best to cut them as short as possible with an electric clipper. The average length of shaft extending beyond the skin surface after careful clipping proved to be 0.5 mm. The animal was then anaesthetized either with 'Dial' or 'Nembutal' injected into the peritoneal cavity and supported in a frame so that the experimental ear was movable and its dorsal surface freely accessible. The ear was positioned so that the great auricular and lesser occipital nerves could be exposed and the surrounding skin raised to form a trough which would retain sufficient fluid for the particular nerve exposed to remain submerged. The ear was anchored in this position by a small piece of cello-tape attached to its tip.

The great auricular and the lesser occipital nerves were exposed as they entered the ear by longitudinal incisions, approximately 2 cm. long, made immediately over them. When exposing the nerves in relation to the vertebral column they were approached in a manner which has been demonstrated in paper II of this series. The epineurium was removed and the nerve trunk flooded with a 0.035 % solution hyaluronidase ('Hyalase' Benger) in normal saline. After a few minutes, a single fasciculus was gently separated from the nerve trunk with fine sharp needles and lifted on to a pair of flamed platinum electrodes lying between 2 and 4 mm. apart. The greatest care was taken not to damage the fasciculus either during separation from the nerve trunk or when lifting it on to the electrodes. Superfluous fluid was removed and the trough filled with warmed (37° C) liquid paraffin (B.P.). The clipped hair shafts were brushed with a no. 0 sable hair brush and only the area over the back of the ear (from which action potentials were evoked) was outlined in coloured ink. In selected cases a single isolated hair or group of hairs emerging from a single orifice anywhere between the margin and the centre of the area outlined were moved rhythmically at a predetermined speed over a predetermined distance by electro-mechanical means (Collin, 1949) and the evoked action potentials recorded photographically. The fasciculus was then excised, and in a number of cases prepared for examination under the microscope; alternate transverse sections either being stained for myelin or impregnated with silver. In the case of the nerves exposed at the base



of the ear, another fasciculus was then freed from the nerve trunk, lifted on to electrodes and the area (back of the ear only) from which action potentials could be evoked outlined in ink of another colour; this fasciculus was also excised and in selected cases prepared for fibre counting. This procedure was continued until the number of areas outlined was as many as could be accurately superimposed in a single experiment.

The second and third cervical dorsal roots were exposed through a mid-line incision and a high cervical laminectomy. The rootlets between the ganglion and the cord were so short that it was found necessary to cut them in turn close to the cord, lift them on to a single electrode and to place the second electrode into surrounding tissues. Care was taken that no muscle intervened between the active and this indifferent electrode. Areas containing hairs subserved by the rootlets were then outlined by brushing in the manner just described.

### HISTOLOGICAL OBSERVATIONS

#### (1) *Whole preparations (unoperated specimens) of dorsal ear skin stained with osmium tetroxide or methylene blue*

To appreciate the significance of the degeneration experiments, it is first necessary to refer to the way in which the nerve bundles enter the ear and how they proceed towards the cutaneous plexus. Pl. 1, fig. 1, was taken from a preparation stained with osmium tetroxide. It shows that the great auricular and lesser occipital nerves enter the ear as flattened bands rather than circular bundles of contiguous fasciculi. The great auricular nerve consists of between 18 and 22 fasciculi and the lesser occipital nerve of between 12 and 15 fasciculi. The great auricular nerve passes down the length of the ear almost to the tip but the fasciculi of the lesser occipital nerve become distributed fairly rapidly.

Immediately after entering the ear, fasciculi leave the great auricular nerve at intervals and pass towards the periphery where they divide, subdivide and inosculate with other fasciculi before entering the dermis to give rise to the cutaneous plexus. Many of the fasciculi reach their destination by pursuing a course closely similar to that of the blood vessels, others take an independent course (Pl. 1, fig. 2). A small number of fasciculi leave the named nerve trunks at intervals and join company with the arteries and veins with which they remain throughout their course and to which they supply terminals at intervals. Throughout the course of the named nerve trunks in the ear, an interchange of axons is continually taking place between the fasciculi to a greater or lesser degree. This is well demonstrated in methylene-blue preparations (Pl. 1, figs. 3, 4).

The fasciculi terminate in the dermis where the individual axons start to divide, subdivide and change direction so that the branches of a single parent axon become distributed over a wide area of skin (Pl. 2, figs. 1-3). The cutaneous plexus, where the branches of neighbouring parent axons interweave, shuffle and re-shuffle, has a fairly characteristic pattern in the rabbit ear. The axons are gathered into bundles arranged in the form of irregular four- or five-sided figures at the angles of which branching and scattering of axons takes place. Hair follicles in groups are most commonly situated at the centre of the figures and the nerves supplying them usually

proceed to their destination from the angles of the figures. Myelinated axons also leave the cutaneous plexus and terminate in unencapsulated endings in the epidermis, dermis and in relation to arterioles and venules. From time to time, single axons are seen pursuing a course in the dermis which is quite independent of the cutaneous plexus (Pl. 1, fig. 5). These axons usually join the plexus at some point where they behave in the same way as the other axons comprising the plexus. The number of myelinated stem axons is related fairly closely to the number and size of the hair follicles per unit area of skin. As has been shown in Paper II, the number, size and arrangement of the hairs (and the follicles from which they arise) varies from region to region of the ear, forming a continuously variable pattern. Nevertheless, from the histological point of view it is certain that even the smallest hair follicles in the skin of the rabbit ear have at least two stem fibres associated with them; the large follicles having between 6 and 10 and the largest between 20 and 30 stem fibres giving rise to separate groups of terminals related to each follicle. The number of myelinated axons giving rise directly to unencapsulated nerve endings per unit area of skin is on the average in the proportion of about one to every four proceeding to hair follicles. This figure, however, takes no account of the amount of collateralization which has taken place in the cutaneous plexus. It merely refers to the number of stem fibres giving rise to terminals in the skin per unit area of skin (Weddell, Pallie & Palmer, 1954; Weddell, Palmer & Pallie, 1955). It is subject to considerable variation from place to place. Broadly speaking, the number of stem fibres giving rise to unencapsulated endings is inversely proportional to the number of large hair follicles in the area of skin concerned.

It is noteworthy that in all the unoperated specimens examined which had been prepared following the use of hyaluronidase, only a very few (1 in 2000) degenerating fascicular nerve fibres were seen throughout the whole extent of the back of the ear. However, no ear was completely free from a degenerating fascicular axon which could be traced for a considerable distance. Degenerating axons were not seen in the cutaneous nerve plexus or on their way to hair follicles. Cf. Weddell & Glees (1941).

## (2) Degeneration experiments

### (a) *Excision of single fasciculi from the great auricular nerve at vertebral column level*

The excised fasciculi were estimated to contain not more than 250, nor less than 50 axons. In two experiments in which the number of axons was counted there were 92 and 116 axons respectively and in the first of these 61 were myelinated axons. A few degenerating nerve fibres were seen throughout the great auricular nerve at the base of the ear. In transverse sections of Marchi preparations the degenerating nerve fibres were seen to be evenly distributed among the fasciculi and evenly throughout each of the 22 fasciculi (Pl. 2, fig. 4). No degenerating nerve fibres were seen in transverse sections from the great auricular nerve at the base of the control ear.

In the methylene-blue preparations a few degenerating nerve fibres were seen in all the fasciculi leaving the great auricular nerve at the base of the operated ear, even extending by inosculation into fasciculi which were themselves chiefly derived from the lesser occipital nerve (Pl. 2, fig. 5). In the control specimens, only an occasional degenerating fascicular axon was seen.

*(b) Excision of single fasciculi at the base of the ear*

The spectrum of fibre diameters in the excised fasciculi when averaged corresponded to that in the lesser occipital nerve (see Paper II). In some of the smaller fasciculi, however, fibres of the largest diameter were sometimes absent. Degenerating nerve fibres at the site of excision were chiefly confined to the fasciculus excised, although in some cases a few degenerating axons were seen in neighbouring fasciculi, indicating the difficulty of separating a fasciculus from the named nerve without severing a few axons passing between fasciculi. The divisions and subdivisions of the divided fasciculus contained an increasing admixture of normal axons as they were traced peripherally (Pl. 2, fig. 6). It was impossible accurately to outline the area of skin overlying the degenerating fascicular axons for, except for a millimetre or so from the point of excision, normal axons had joined the damaged fasciculus and vice versa. Further, degenerating axons could be traced from the damaged fasciculus for several centimetres beneath the skin before they entered the dermis. The overall picture under a 16 mm. objective shows that there is a zone containing a maximal number of degenerating fascicular axons which grows rapidly more normal as the axons fan out into neighbouring fasciculi and/or pass into the dermis. The area involved varies from fasciculus to fasciculus independently of the number of axons which they contain. Some fasciculi are composed of axons which enter the dermis and join the cutaneous plexus within a few millimetres of the point of damage. Others travel as much as 15 mm. before many of their axons enter the dermis or join neighbouring fasciculi among which they may travel for a further distance of up to several centimetres before they in turn enter the dermis. The area of skin, in which degenerating axons in the cutaneous plexus and those approaching hair follicles are seen, is commonly oval in shape (when the fasciculus excised subserves only the dorsum of the ear) and the number of degenerating axons is greatest at the centre of the area involved. The manner in which the fascicular axons are distributed within the various areas of skin which they subserve differs considerably from fasciculus to fasciculus. Broadly speaking, it is directly related to the way the fasciculus reaches the area concerned. As noted above, the majority of axons may join the cutaneous plexus more or less simultaneously after proceeding a short distance only. In such a case they become rapidly and fairly evenly intermingled with normal axons and occupy a roughly oval skin area, the long axis of which commonly lies in the long axis of the ear. The largest proportion of degenerating nerve fibres lies towards the centre of this zone and the number tails off gradually towards the periphery. Here the proportion declines relatively abruptly and irregularly.

In other cases, the area of skin in which the greatest number of degenerating axons is seen is not a compact, relatively small ( $3 \times 2$  cm.) oval, but a much larger area, the peripheral boundary of which is either hard to demarcate or reaches the margin of the dorsal ear skin. It may be as large as  $4 \times 8$  cm. In general, in the large areas the degenerating axons are more widely dispersed among the normal axons but this is not always the case. As already stated, the number but not necessarily the diameter of the degenerating axons (nerve fibres of all diameters appear to be indiscriminately scattered throughout the affected zone) declines slowly but steadily towards the periphery of the affected area until a very irregular boundary is reached on one side



of which there are a considerable number of degenerating axons but on the other side of which only a few degenerating axons supplying hairs are seen. The boundary, however, is not sharp, for after excision of a single fasciculus a few degenerating axons ranging in diameter from the smallest to the largest, can be seen patchily distributed over almost the whole extent of the ear. Some of the more widely distributed and smaller of the degenerating axons do not appear to terminate in relation to hair follicles but some can be seen to supply them. In the central zone it is very difficult to distinguish axons which terminate in relation to the dermis and epidermis from those which terminate in relation to hairs. All that can be said with certainty is that a proportion of the myelinated axons end in relation to structures other than hair follicles.

A feature in these observations which requires emphasis is the widespread and variable, though minimal, damage which ensues when even a small fasciculus is excised from the named nerve at the base of the ear (Pl. 2, fig. 7). This leads to almost insurmountable difficulties when attempts are made accurately to outline areas containing degenerating axons from the surrounding skin.

In some cases, it is possible to outline a circumscribed area under low magnification (25 mm. objective) which has an apparently sharp border. This 'border' is due to the fact that when the proportion of degenerating axons in the cutaneous plexus has fallen below a certain number (which varies in relation to many factors such as the depth of staining and thickness of the skin) their presence can only be distinguished under higher magnification. The appearance of a sharp border may also be due to the greater depth of focus available under low magnification. This makes it impossible to distinguish degenerating nerve fibres in the fasciculi from those in the cutaneous plexus; both come into focus together, and thus an apparently clear-cut but composite area may be outlined.

So far, we have confined our observations to skin from the dorsum of the ear. However, the greater part of the skin over the ventral surface of the ear is also supplied by the great auricular and lesser occipital nerves. A number of fasciculi contain axons which enter the cutaneous plexus and supply hairs in an area adjoining the margin of the ear. In such instances, the fasciculus continues round the margin, giving rise to axons which enter the cutaneous plexus to supply hairs on the ventral surface. The number of axons in the cutaneous plexus in ventral ear skin is on the average less than in dorsal ear skin, particularly towards the centre. However, since the general pattern of innervation appeared in no way different in skin from either surface, no quantitative studies were attempted in relation to ventral ear skin. No nerve fibres have ever been seen to pierce the auricular cartilage to reach the skin over the ventral surface of the ear.

#### ELECTROPHYSIOLOGICAL OBSERVATIONS

##### (1) *Areas subserved by fasciculi entering the base of the ear*

It was not possible to obtain records from all the fasciculi composing the great auricular and lesser occipital nerves in a single animal. The nerves together contain a large number of fasciculi (which, in the animals examined, varied between 30 and 37) and each time a fasciculus is dissected free, the probability is that the main

nerve trunk will suffer some slight damage, so that there is always a limit to the number of areas which can be accurately outlined. Moreover, fasciculi from the named nerves subserving hairs at the margin of the ear pass round to the front to supply hairs over the ventral surface. Some fasciculi subserve hairs over a wide area on the ventral surface and in positions which are difficult to outline. For this reason, we confined our observations to areas of skin over the dorsal surface of the ear which could be measured reasonably accurately. In addition, all our calculations have been made in such a way that the fact that some of the fasciculi from which we have recorded subserve hairs on the ventral as well as the dorsal surface of the ear is irrelevant.

In two animals, records were made from as many fasciculi as possible to obtain a general picture of the size, shape and relationship of the areas over the dorsum of the ear. In the majority of animals (15) a few of the fasciculi were selected at random from either nerve and the area of skin which they subserved on the dorsum of the ear outlined. The finer the fasciculus the greater the size of the action potentials relative to the noise level; thus, the easier it was to map the area by brushing the hair stumps and observing (chiefly by listening to) the electrical responses from such fasciculi. The area is marked out by a smooth uninterrupted line, which is in fact drawn in between a series of points very closely scattered on either side of it. The appearance of a clearly defined border is therefore misleading. The hair stumps, when brushed, either give rise to action potentials which can be clearly recorded or, apparently, to no action potentials whatever, so that the borderline of the area, though sometimes irregular, is always definable. Attempts were made on numerous occasions to evoke action potentials by brushing from isolated hair stumps far outside the area outlined on the dorsum, but always without success. These findings are difficult to reconcile with our histological observations and, for this reason, more than usual care was taken to ensure that our technique was impeccable and that our recording equipment was of first quality. In particular, special care was taken to avoid damaging the fasciculi which would have given rise to spurious records unrelated to propagated disturbances from the hairs stimulated. In addition, after an area subserved by a fasciculus was outlined, the fasciculus in question was crushed distal to the recording electrodes. In every case, this blocked conduction and no evoked action potentials were subsequently recorded in response to brushing anywhere over the ear, thus confirming that the recorded response was in fact evoked from the hairs stimulated.

More detailed analysis of our records showed that action potentials of large size were sometimes found by stimulating hairs towards the centre of the active zone whilst stimulation towards the periphery only gave rise to small action potentials. This was by no means always the case. In some instances, the size of the potentials was relatively even throughout the active zone, although at the actual margin small potentials were more common than large potentials. It was also observed that large hairs usually gave rise to larger action potentials than small hairs but, again, this was not invariably the case; on rare occasions large potentials were obtained close to the margin of the area subserved. In many instances, the larger action potentials had a complex wave form, occasionally a simple wave form. The smaller action potentials were commonly simple diphasic spikes.

In view of these observations, areas subserved by very fine fasciculi, containing between 20 and 40 myelinated axons, encountered towards the tip of the ear, were outlined. In the majority of such areas, hairs in the centre of the zone gave rise to relatively very large, usually simple, diphasic spikes. The size of the spikes diminished rapidly as hairs towards the margin were stimulated. Hairs giving rise to very large action potentials were never encountered at the margin of the area. In addition, very few spikes of complex wave form were encountered from hairs in these areas.

When we attempted to correlate an area mapped electro-physiologically with the degeneration resulting from excision of the fasciculus, we found that in every case the area outlined by the evoked action potential technique was only 1 or 2 mm. smaller than that judged under the lower power of the microscope to be the margin where the greatest transition took place. However, it appeared certain that, under the conditions of our experiments, we were unable to record activity from hairs supplied by single axons of large or small diameter situated beyond a certain distance from the centre of maximal response.

In an experiment in which records were obtained from 18 fasciculi at the base of the ear in a single animal (see Table 1) it was found that as many as 6 fasciculi innervated a common zone of skin (Text-fig. 1). In other experiments in which the number of areas outlined was less, the number of fasciculi subserving a common zone was correspondingly less. The figures obtained from 48 fasciculi sampled in five animals are set out in Table 1. It can be seen that, as the number of fasciculi

Table 1

Zones subserved by fasciculi (actual area and % of total area mapped)

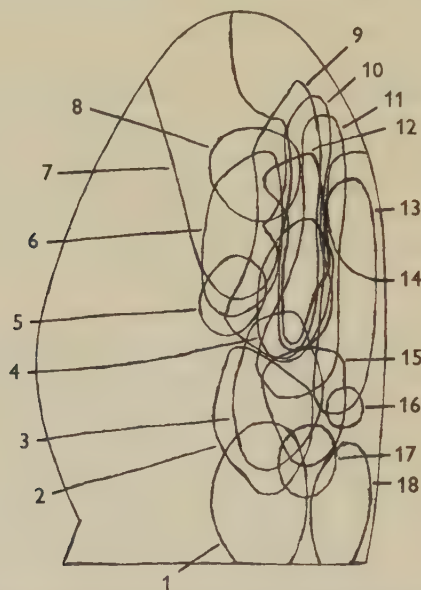
Rabbit ear	No. of fasciculi sampled	Total area mapped in cm. <sup>2</sup>	Two or more		Three or more		Four or more		Five or more		Six	
			cm. <sup>2</sup>	%	cm. <sup>2</sup>	%	cm. <sup>2</sup>	%	cm. <sup>2</sup>	%	cm. <sup>2</sup>	%
C	4	11.7	4.4	37	1.3	11	—	—	—	—	—	—
A	6	10.8	5.8	54	1.4	12.7	—	—	—	—	—	—
D	8	12.7	4.1	32	1.5	11.4	0.51	4	0.05	0.4	—	—
E	12	17.7	12.3	70	7.4	42.0	0.82	4.6	—	—	—	—
G	18	14.1	6.8	48	4.3	30.0	2.1	14.8	0.81	5.8	0.07	0.5

examined increases, the number of areas found to overlap with one another also increases. The percentage of the area examined which receives the greatest number of fasciculi, however, tends to grow rapidly smaller. Thus, Table 1 shows that in rabbit ear (A) 6 fasciculi were sampled, the total area mapped was 10.81 cm.<sup>2</sup> and the maximum number of fasciculi subserving a common zone of skin was 3, the area of the zone being 1.37 cm.<sup>2</sup>. This zone was 12.7 % of the total area of skin mapped. In rabbit ear (G), on the other hand, in which areas subserving 18 fasciculi were outlined, 6 fasciculi were found to subserve a common zone of skin, the area of which was only 0.07 cm.<sup>2</sup> or 0.5 % of the total area mapped. Thus, when a large number of fasciculi are sampled from a single rabbit ear, it is seen that there is a marked diminution in the size of the common zone subserved by the largest number of fasciculi. It is therefore reasonable to assume that if, under these conditions, a larger number of fasciculi were sampled, the number found to be subserving a



common zone of skin of any appreciable size would not greatly exceed six. On the other hand, our histological observations suggest that the areas outlined electrophysiologically are deceptively small. It is thus possible that the figure six is on the low side.

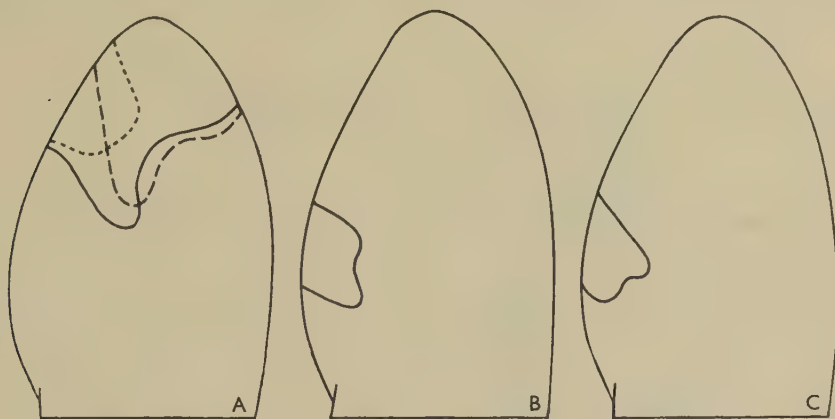
Table 1 also shows that there is no direct numerical relationship between the number of fasciculi examined and the percentage of the total area of dorsal ear skin which they subserve. This is of course due to the difference in size, shape, as well as to the location of the areas subserved by each fasciculus, an observation which has also been confirmed histologically. It is equally clear that areas related to different fasciculi in the same ear overlap extensively; indeed, on occasions, one area may lie completely within another (Text-fig. 2A). The areas form a pattern of such complexity that it is certain that a unit area of skin from anywhere on the ear will not be related to a fixed number of fasciculi.



Text-fig. 1. The relationship of the areas (outlined by brushing hair stumps) subserved by 18 fasciculi, four of which were from the great auricular nerve and 12 from the lesser occipital nerve at the base of a single rabbit ear. Six fasciculi subserve a common zone of skin.

The size of the areas on the back of the ear subserved by each of 67 fasciculi in twelve rabbits were measured, and since the actual size of the ears involved was known, it was possible and permissible (see Paper II) to scale these areas in relation to an ear of an 'average' size (31 cm.<sup>2</sup>). The range of sizes so obtained has been plotted against the number of fasciculi examined in the form of histograms (Text-fig. 3). These indicate that there is a wide scatter about a mode and that the fasciculi in the lesser occipital nerve are generally associated with smaller areas than those in the great auricular nerve. At the same time, it was noted that the particular size and shape of the areas mapped in the same region of the ear did not vary greatly from animal to animal (Text-fig. 2B, C).

In view of this observation, it was considered permissible to use the figures we had obtained to calculate the average number of dorsal root axons subserving a common zone of dorsal ear skin. We did this by calculating from our histograms the area of skin over the dorsum of the ear subserved by each of the average number of fasciculi



Text-fig. 2. A: The relationship of the areas (outlined by brushing hair stumps) subserved by three fasciculi of the great auricular nerve as they entered the base of a single rabbit ear. Note the extensive overlap. B and C: Areas subserved (outlined by brushing hair stumps) by single fasciculi of the auricular nerve in different rabbit ears. Note the similarity in shape and orientation of the areas.

Table 2

Fasciculus serial no.	Area (cm. <sup>2</sup> )	Number of nerve fibres	
		Total	Myelinated
1	2.7	104	88
2	2.1*	378	244
4	1.4*	220	150
5	0.4	91	60
6	2.3	281	199
7	2.9*	469	316
8	3.9*	247	186
9	2.7*	174	120
10	2.0	142	100
12	1.9*	160	106
13	2.1*	245	162
14	0.9	244	145
15	2.9	131	95
16	2.6	197	141
17	2.7	198	126
18	0.7	73	51
19	4.0*	443	272

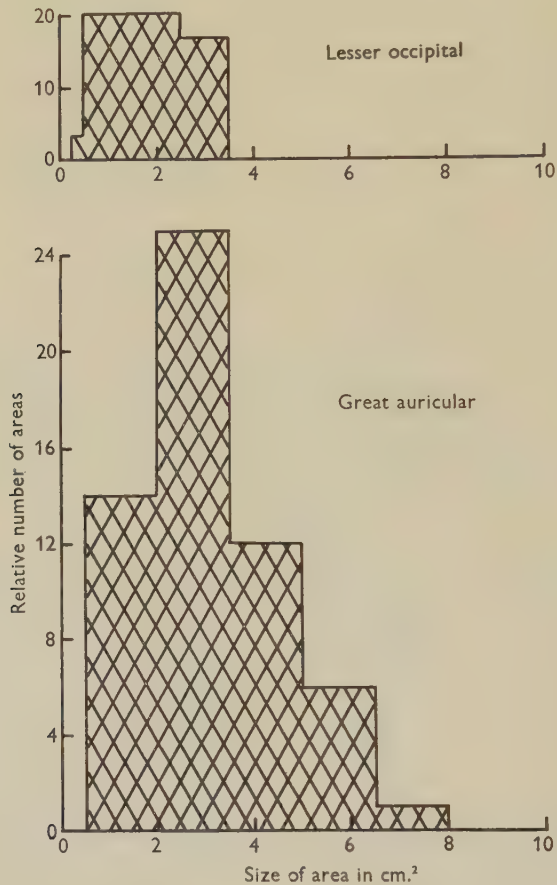
\* Indicates fasciculus also supplying an unknown area of skin on the front of the ear.

contained in both the great auricular and lesser occipital nerves. This proved to be four times greater than the total area of dorsal ear skin. In the hope that this average figure might help us to visualize the anatomical arrangements more clearly we proceeded as follows:

In seventeen instances the number of axons in the fasciculi subserving measured areas of dorsal ear skin were enumerated. They are shown in Table 2, scaled in relation to an ear of average size (viz. 31 cm.<sup>2</sup>). It will be noted that some fasciculi

supplied hairs over a variable area of ventral ear skin, the extent of which was not measured. In these instances, the figures obtained have a relative, rather than an absolute value.

The number of myelinated nerve fibres in the fasciculi were next plotted against the areas which they supplied in two series: (1) those concerned solely with the



Text-fig. 3. Histograms which show the relative number of areas of a particular size subserved by single fasciculi in the named nerves at the base of the ear.

dorsum of the ear, and (2) those also concerned with unknown areas of skin over the front of the ear. This showed in both series that, although there is a general tendency for the larger fasciculi to subserve larger areas, the scatter is very great indeed. In other words, there is not a linear relationship between them. On the contrary, casual inspection of Table 2 clearly indicates that it is impossible to predict from the number of myelinated fibres in a given fasciculus the size of the skin area which it will subserve. An attempt was made to account for this on the basis of the varying number and size of the hairs in the different areas of skin in question but, as might

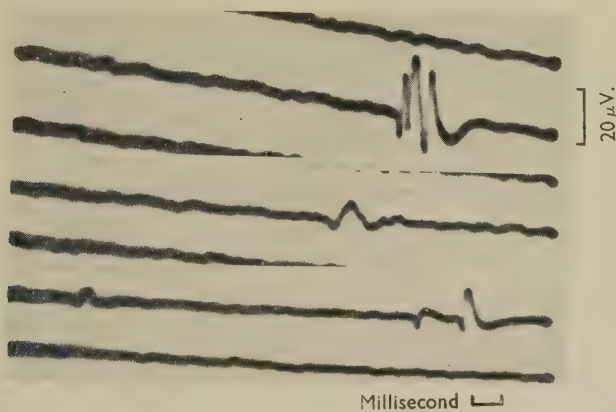


have been predicted on the basis of the observations in Paper II of this series, no simple relationship was found to exist. We are thus forced to the conclusion that, although on the average each unit area of skin may be subserved by four dorsal root axons, it is highly probable that there is a very complicated patterned arrangement which ensures that in certain skin areas, some hair follicles are innervated by fibres derived from more than six dorsal root axons, others by less, but, judging from our histological observations, none by less than two.

(2) *Analysis of the number of hairs giving rise to action potentials within the area subserved by a given fasciculus*

After outlining an area subserved by a fasciculus, a number of fine straight lines, some of which passed approximately through the geometrical centre, were drawn across it in indian ink. Each isolated hair or hairs emerging from a single orifice on each of these lines was then stimulated rhythmically by striking it with a rigidly mounted thorn, the movements of which could be controlled within fine limits. On each occasion the stimulus was so adjusted that only the selected hair or hairs were seen to move under the microscope. The results are set out in Table 3.

From this it can be seen that the number of active hairs ranged from 57 to 100 % in the case of any particular line in any particular area. The size and complexity of the action potentials evoked varied from hair to hair (Text-fig. 4) within the area

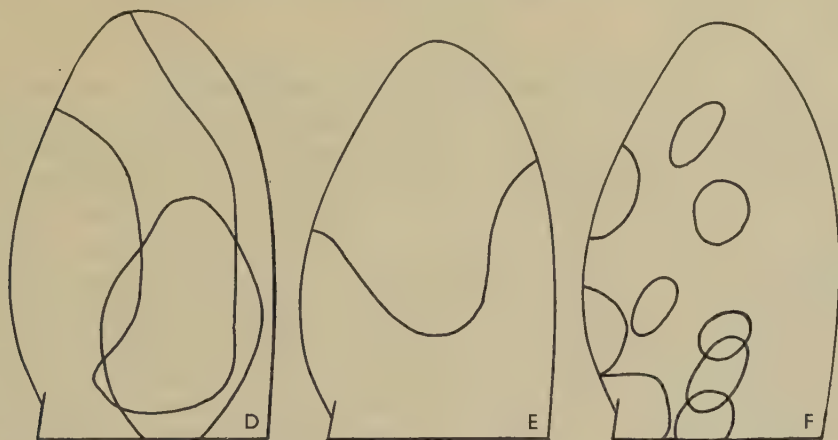


Text-fig. 4. Action potentials evoked by stimulation of three separate hairs emerging from different orifices in the skin within an area subserved by a single fasciculus at the base of the ear. Note the difference in amplitude and wave form.

stimulated in a random manner. The spikes from hairs at the margin of the area outlined are, however, usually smaller than those obtained from elsewhere. The presence of inactive hairs at the margin is probably no more than an indication that the margin is an irregular one. It is also clear that inactive hairs are irregularly distributed on the course of some lines, particularly those crossing large areas. This suggests that the nerve fibres contained in a fasciculus are not always uniformly distributed within the area subserved by the fasciculus. These observations are in accord with our histological findings following the excision of fasciculi.



Our electro-physiological observations taken together (Table 3 and Text-fig. 1) suggest that under appropriate conditions each and every hair or hairs emerging from a single orifice will give rise to an action potential which can be recorded. This confirms our histological observation that all the hair follicles over the dorsum of the ear are innervated by myelinated nerve fibres.



Text-fig. 5. D and E: The areas suberved (outlined by brushing hair stumps) by three fasciculi of the great auricular nerve near the vertebral column. These fasciculi contained approximately the same number of dorsal root axons as the fasciculi subversing the areas shown in Text-fig. 2A-C. F: The areas suberved (outlined by brushing hair stumps) by dorsal rootlets of the third cervical nerve between the dorsal root ganglion and the spinal cord. The rootlets were comparable in perimeter to the fasciculi sampled elsewhere. Note the size of the areas as compared with those in D and E and in Text-fig. 2A-C.

### (3) Areas suberved by fasciculi in the nerve trunks near the vertebral column

These fasciculi were found to subserve relatively much larger areas of dorsal ear skin, although the number of nerve fibres which they contained did not exceed those found to be present in fasciculi at the base of the ear. For instance, in two fasciculi the total number of nerve fibres was found to be 92 and 116 and they were related to 14 and 18 cm.<sup>2</sup> (scaled to an ear of 31 cm.<sup>2</sup>) of skin respectively. The areas are shown in Text-fig. 5D, together with another area, Text-fig. 5E, in which the fasciculus was of the same order of size but in which a fibre count had not been made. Two other interesting observations emerged: 1, the size and complexity of the action potentials evoked from these areas were in every case less than those evoked when recording from fasciculi at the base of the ear. 2, Action potentials could be evoked from less than one-third of the hairs (qualitative estimate), evenly dispersed throughout the mapped area.

### (4) Areas suberved by fasciculi in the dorsal roots

Three animals were used in these experiments and a total of nine areas outlined on the dorsum of the ear. For technical reasons, only the third cervical nerve root was sampled in detail in each animal. It was not possible to obtain suitable specimens of nerve for fibre counting, but under the dissecting microscope ( $\times 10$ ) their sizes



appeared to be of the same order as those used in the previous experiments. The areas mapped are shown in Text-fig. 5F. The areas were small in every case and comparable to those obtained by sampling fasciculi as they entered the base of the ear (see Text-fig. 1). Owing to the different recording technique no attempt was made to make an analysis of the form or size of the action potentials in these experiments.

#### DISCUSSION

In the first paper in this series we showed that about 9000 nerve fibres enter the rabbit ear, of which about 6500 are myelinated and are of dorsal root or cranial sensory nerve origin. At the same time, we pointed out that it was not possible to define the pathways pursued by individual axons in relation to one another in the named nerves with the methods at our disposal. The observations reported in this paper take us further. By a combination of degeneration and electro-physiological experiments, we have shown that a single large hair or group of hairs emerging from a single orifice may be supplied by as many as six separate dorsal root axons and that even in the case of the smallest hairs not less than two dorsal roots are involved. But we also know from degeneration experiments that the evoked action potential technique, from which we deduced the number six, is likely to give us a deceptively small number; indeed, we know that the largest hair follicles may be supplied by as many as twenty-six stem axons (Weddell, Pallie & Palmer, 1954). For these reasons, it is very probable that hairs in some areas of skin are supplied by more than six dorsal root axons.

The nerve fibres leaving the hairs enter the cutaneous nerve plexus whence they find their way into fasciculi and so into the named nerve trunks to reach the dorsal root ganglia. We have demonstrated that, on their way, individual axons which terminate in close proximity to one another part company so that axons from a given zone of skin become scattered evenly throughout the named nerve trunks. It is this arrangement which determines that interruption of up to 25 % of the named nerves does not lead to complete denervation of any part of the skin but only to an overall diminution in the number of nerve fibres throughout the area of skin supplied by the named nerve (Weddell, 1941). Thus, less than total interruption of the nerve will lead to a general diminution of sensory acuity rather than to a change in the fundamental nature of the message received from the hairs.

We have also demonstrated that between the dorsal root ganglia and the spinal cord there is a complete re-orientation of the axons so that they are no longer arranged in a random manner but become regrouped in such a way that the axons terminating in close proximity to one another in the periphery are also closely related to one another as they enter the cord. Thus a single fasciculus in the periphery innervates a relatively small, and more or less sharply defined, area; a single comparable fasciculus in the nerve trunk at the level of the vertebral column innervates a large less well-defined area (often comprising more than half the ear); and a single comparable fasciculus between the dorsal root ganglion and the spinal cord again innervates a relatively small and more or less sharply defined area.

It has been noted that hairs in the rabbit ear (which are of different sizes and arranged in a highly complex pattern, see Paper II) are all supplied by myelinated

nerve fibres but we have also demonstrated that not all myelinated stem axons terminate in relation to hairs. It is certain that collaterals from parent nerves supplying hairs do not give rise to collaterals ending in unencapsulated nerve endings (Weddell, 1941, Weddell, Pallie & Palmer, 1954). Thus, not all the myelinated dorsal root axons entering the ear subserve hairs, and our histological observations suggest that possibly one-quarter end elsewhere than in relation to hairs. We have estimated that there are about 5000 myelinated dorsal root or cranial sensory nerve axons available to innervate about 100,000 hairs, and we know that each hair on the dorsum of the ear is supplied by at least two, some by six cervical dorsal root axons. It can therefore be deduced that each myelinated axon entering the ear will send branches to between 40 and 120 hairs on the back of the ear. This is a conservative estimate, for our histological observations suggest that the maximum figure may well be greater.

Confirmation that the figures deduced from the overlap experiments give us a reasonable idea of the number of hairs supplied by a single dorsal root axon is given by observations made in relation to a quite separate series of experiments. We have demonstrated that, to all intents and purposes, 100 % of the hairs in an area subserved by a fasciculus at the base of the ear are active if allowance is made for the limitations imposed by the nature of the experiments. We have also shown that the sum of the areas of dorsal ear skin subserved by each of the fasciculi entering the ear is four times greater than the total area of skin covering the dorsum of the ear. These observations, when taken together, suggest that the average number of dorsal root fibres supplying each hair is four. It is significant that this figure is the exact mean of that suggested by the overlap experiments.

We are now in a position to integrate some of the observations which we have made in each of the foregoing papers. In the rabbit's ear, the hairs vary in size and are arranged in a complicated and continuously variable pattern which ensures that any contact stimulus must strike a number of hair follicle groups. The axoplasmic terminal filaments ending in relation to a hair follicle are arranged in two quite separate groups, lying in different tissue layers, one within the other, parallel to and encircling the shaft respectively (Weddell, Pallie & Palmer, 1954). In addition, each hair is supplied by a variable number of dorsal root axons (in excess of two) roughly related to the size of the follicle from which it springs. Over and above this is the orderly but highly complex anatomical course and patterned arrangement of the axons lying between the hairs and the spinal cord.

It has been demonstrated that on the average 80 hairs are subserved by a single dorsal root axon. From this it follows that once a nerve impulse from one of those hairs reaches the parent dorsal root axon, it is no longer specifically related to the particular hair from which it arose. In other words, an action potential travelling up a given dorsal root axon may have originated from any one of a large number of hairs widely separated from one another.

However, each hair is on the average innervated by four dorsal root axons. Thus, nerve impulses will travel up four axons within the named nerve trunk when a single isolated hair is stimulated. (This is of course a special and over-simplified condition unlikely to exist in everyday life.) Although the four action potentials are dispersed at random in the named nerve trunk they become re-orientated between the dorsal

root ganglion and the spinal cord so that the image transduced in the periphery is to some extent at least, reformed at this, the first level of integration. This anatomical arrangement alone must determine, at least in part, why punctate stimuli in the spinal animal elicit reflex responses patterned in relation to the site of stimulation. Nevertheless, the anatomical arrangement by itself cannot account for precise localization since each of the four impulses could have come from any one of 320 hairs in four distinct regions.

Our histological observations, however, make it clear that the closer a particular hair is to its parent dorsal root axon, the more quickly an impulse originating from that hair will begin its course to the spinal cord. For this reason, the temporal pattern of the four action potentials is likely to be unique, for the hairs subserved by a single dorsal root axon are all situated at varying distances from the parent nerve fibre at the end of nerve fibres whose diameters decrease in the cutaneous plexus as they approach their termination (Weddell & Glees, 1941). Moreover, the diameters of the dorsal root axons subserving hairs themselves vary over a wide range. Thus, localization is likely to depend not only upon the spatial but also upon the temporal relationship of the impulses which reach the central nervous system.

It follows, then, that a relatively small number of dorsal root nerve fibres will be able to convey a very large number of different uniquely patterned action potential sequences. Thus, there exists a system for the transmission of information containing enough detail to allow of a precise response even in respect of the stimulation of a single hair in the rabbit ear. In the case of the whole ear, the information available to the central nervous system must be very large indeed.

In view of these arrangements, it is impossible to regard either a single nerve fibre or a single nerve ending related to a single hair as a primary sensory unit for under no natural circumstances is only a single nerve ending or only a single nerve fibre ever stimulated. Moreover, we have shown that it is possible to occupy each and every dorsal root axon subserving hairs with action potentials evoked by selectively stimulating hairs situated in an area occupying less than one-quarter of the total skin surface of the ear, and this can only mean that information relative to contact with hairs cannot be related directly and exclusively to the number of dorsal root and cranial sensory nerve fibres supplying the ear.

When the rabbit's ear is touched, the stimulus is translated by the hair follicle transducers into a pattern of impulses which are transmitted by the whole, or a large percentage of, the myelinated nerve fibres in the named nerves and impressed on the central nervous system as a spatio-temporal pattern of impulses, dependent upon the number and position of the transducers stimulated. The ability of a system of the kind which we have demonstrated to transmit a given amount of information does not, as we have already pointed out, depend simply upon the number of nerve fibres available in the nerve trunk. It must depend upon the relationship of their conduction velocities, i.e. their diameters. It must also depend upon the number of transducers available to each nerve fibre as well as to their size, arrangement and transducing properties.

So far, we have discussed the anatomical arrangements which determine that when the hairs of the rabbit ear are stimulated, a spatio-temporal pattern of nerve impulses representing those aroused in the transducers (hair follicles) by the stimulus



is impressed upon the central nervous system. We do not know the fate of the impulses after they have entered the spinal cord; it is of the greatest interest, however, to find that Adey, Carter & Porter (1954) have shown that a pattern such as we have predicted reaches the cerebral cortex in the rabbit. They state: 'In both phalanger and rabbit the afferent volleys from homolateral and contralateral limbs show constant patterns of temporal dispersion over the cortical surface in the first somatic area, and these are clearly different for the two limbs. The evoked potentials from the homolateral limb show an essentially constant latency across the responsive area, whereas the latency of contralateral responses decreases rapidly from the periphery to the centre of the area. In stimulation of the prehensile forelimb of the phalanger, the total responsive zone in the contralateral first somatic area remains constant in size and disposition from stimulation of widely separated points in the digits and distal forearm. However, the cortical point within this area yielding the most rapid responses shows constant alterations in position with changing sites of peripheral stimulation, and it is suggested that *perception and localization may occur in terms of the disposition of the point of shortest latency within an activated area of essentially constant site and dimensions.*'

Because of the overlap between areas subserved by adjacent fasciculi at the base of the ear and at their entrance into the spinal cord, excision of a single fasciculus at either of these levels leads only to an area of diminished sensibility (hypoesthesia) but not to an area of insensitive (anaesthetic) skin. For this reason it is difficult to understand why adjacent axons in the periphery part company and become dispersed in a random manner in the named nerve trunks when they regroup once again before entering the spinal cord. A possible explanation is suggested by the observations of Katz & Schmitt (1940). They have shown that under certain circumstances 'during the passage of an impulse in one fibre (from the limb nerve of the crab *Carcinus maenas*), subthreshold excitability changes take place in an adjacent fibre'. In addition, they have shown that: 'When impulses are set up simultaneously in both fibres, a mutual interaction takes place producing various combinations of speeding and slowing, depending upon the phase relationship between the two impulses. If the impulses are advancing entirely "in step", their local action currents interfere with each other, and the propagation velocity is reduced. If one impulse slightly precedes, it accelerates the conduction rate of the lagging action potential. This effect leads to a "synchronization", and to equalization of speeds of the impulses, if their individual velocities differ only slightly.'

If comparable interaction between adjacent axons in mammalian nerve trunks were to take place, then the spatio-temporal pattern from the periphery might undergo distortion unless axons subserving adjacent peripheral transducers were widely separated from one another. The arrangement which exists would certainly limit interaction of this kind. Nevertheless, it is difficult to conceive what mechanisms permit this arrangement to take place in the course of development.

It has long been known (Adrian, 1931; Zotterman, 1939) that if action potentials are recorded from small nerve bundles just before they enter the skin, a characteristic pattern of activity is obtained by a brush stroke carried across the area which they subserve. We have confirmed the observations that, in general, action potentials in the centre of the area stimulated are larger than those obtained towards

the periphery. Adrian suggested that this might be due to the existence of 'protopathic' and 'epicritic' touch fibres. Zotterman, however, noted that the smaller action potentials at the periphery were also obtained by the lightest touching of the hairs in the centre of the area, in the after-discharge following a firm stroke and during burning. He believed that these action potentials might be concerned with the sensations of tickling or itching. We have shown histologically that in each of the peripheral bundles innervating a more or less sharply defined area, there are more axons innervating hairs lying in the centre of the area than axons innervating hairs lying in the periphery. Thus we suggest that the large action potentials in the centre of the area stimulated are due to a summation effect. Many fibres will be active in this region and fire synchronously. Moreover, the fibres from the central zone will be adjacent to one another at this level, and any possible lack of synchronization may well be compensated for by the phenomenon described by Katz & Schmitt. If we are correct, then a somewhat similar picture to that obtained in the extreme periphery is likely to be encountered when recording from a single fasciculus between the dorsal root ganglia and the spinal cord. We have confirmed that this is so in the cervical region in the rabbit. Kuhn (1953), recording from dorsal rootlets in the lumbar region of the cat, has also noted a similar picture in response to tactile stimulation. We should also expect, on the basis of this argument, that only small action potentials would be evoked when recording from fasciculi equivalent in size dissected from the named nerve trunks and there is evidence that this is the case. The alternative explanation that in each peripheral fasciculus only axons of large diameter innervate the hair follicles in the centre of the area is untenable. For one thing, such a picture is not seen under the microscope. In the second place, if this explanation were correct, each hair in the centre of every area subserved by every small nerve bundle in the periphery would have to subserve each hair. In other words, 200,000 (twice the number of hairs in the rabbit ear) myelinated axons of two different diameters would be concerned with innervating hairs in the rabbit ear; we have, of course, demonstrated that this is not the case.

It will be recalled that the purpose of these investigations was to study the innervation of hairs on a quantitative basis to determine whether the anatomical arrangements were such that stimulation of a hair in a specific manner was likely to give rise to the discharge of nerve impulses of a characteristic spatio-temporal pattern. In view of our observations, this question can now be answered in the affirmative in the case of the rabbit ear. Indeed, we have shown that the hairs, together with the nerves which supply them in the rabbit ear, constitute very much more than a simple binary system (see Appendix) for the presentation to the spinal cord of information relative to contact.

At least one-third of the myelinated nerve fibres give rise to arborizations of free, naked, axoplasmic filaments which end among the cells of the epidermis and among the structures which compose the dermis. The arrangement of these terminal arborizations is very complex but degeneration studies suggest that the patterned arrangement of the terminals and the nerves which subserve them is not very different from those subserving the hairs. In view of the fact that the neuro-histological picture in the human pinna resembles that in the rabbit (Sinclair, Weddell & Zander, 1952) and in the case of hairy skin elsewhere in the human body

(Weddell, Pallie & Palmer, 1954), it is perhaps not out of place to discuss the bearing of the observations which we have just reported on the mechanism of cutaneous sensibility in man.

It is possible to arouse all four of the so-called 'primary modalities' of sensation (i.e. touch, warmth, cold and pain) from the human pinna. If we assume that the hairs subserve touch, then the myelinated and unmyelinated nerve fibres subserving the unencapsulated nerve endings in the skin must (on the basis of the classical theory of cutaneous sensibility) be divided into three groups, one for cold, one for warmth and one for pain, the spatial distribution of each group being approximately the same.

The unquestionable validity of the 'all-or-nothing' law in respect of the activity of individual stem nerve fibres (and, presumably, of integral multiples of individual nerve fibres) has led to the acceptance by most observers of a simple point to point system of information, postulated for each of the four 'modalities' of cutaneous sensibility by von Frey. This was an elaboration of the 'laws' of specific nervous energies enunciated by Müller in particular relation to the special senses (Müller, 1842). These are generally taken to mean that stimulation of a given sense organ gives rise to a particular sensation and to no other (Fulton, 1943). Further codification of Müller's 'laws' has come to mean that there are four simple units of point to point connections: one for touch, one for warmth, one for cold and one for pain. There are, according to this theory, only two possible states in each 'modality': active, i.e. giving rise to a sensation, or inactive, i.e. arousing no sensation. A tactile stimulus, for example, selectively excites only 'touch' receptors and hence initiates impulses travelling up the fibre attached to the touch receptor in the appropriate pathway to the appropriate cells in the higher centres. Since action potentials are demonstrably non-specific, specificity has been attached to the information system. In the words of Walshe (1942), in a critical review of cutaneous sensibility, 'the individual nerve impulse is not of primary importance in the determination of the final sensory effect, but that the sensory pathway *as a whole* has components specific for each mode of sensibility. Each component in the pathway contributes an element to this specificity: the end-organ with its selective excitability... the fibre itself in virtue of its size, ... also imprints specific effects upon the impulses that traverse it; while, finally, the central destination of each specific sensory group of fibres, and the variations in the central excitatory states, act as the final determinants of the sensory effects. There is, therefore, a mode of specific nerve energy, not resident in the single impulse, but a product of the combined activity and morphology of each component of the sensory path.' In other words, the classical view attributes the existence of the four primary modalities to the existence of four separate systems of nerve fibres connected point to point and represented in the skin ubiquitously.

However, information from a number of different sources suggests that the classical view is unacceptable. For instance, there is growing evidence that the 'modality' of touch can be subserved, not only by hair follicles and encapsulated nerve endings but also by unencapsulated nerve endings (Waterston, 1923; Tower, 1940; Bishop, 1943; Lele, 1954; Lele, Weddell & Williams, 1954). It has also been inferred that unencapsulated (free) nerve endings subserve the modalities of warmth and cold (Sinclair *et al.* 1952; Hagen, Knoche, Sinclair & Weddell, 1953) as well as



the 'modality' of pain for which there has long been a large body of evidence (Weddell, 1953; Weddell, Pallie & Palmer, 1954). For these reasons, Lele *et al.* found it necessary to re-examine the implications of the 'law of specific nerve energies' in relation to a system of information. They have suggested, as the result of observations on the effects of heat transfer, that the specific pattern of impulses elicited by a stimulus depends ultimately upon the way in which the stimulus affects the skin and not upon the particular nerve fibre stimulated. The sensations of warmth and cold are most easily explicable on the basis that they are the products of heating or cooling the surface of the skin upon uniquely disposed but non-specific free nerve terminals situated in different layers. The specificity of the sensation of touch is explained on the basis that the response to deformation of morphologically indistinguishable free nerve endings is the same whether they are encapsulated (Alvarez-Buylla & Ramirez de Arellano, 1953; Gray & Sato, 1953) or free (Adrian, 1931; Tower, 1940): i.e. in both cases they give rise to a rapidly accommodating burst of action potentials. It has been proposed (Lele *et al.*, 1954, and Tyrrell, Taylor & Williams, 1955) that the response of free nerve terminals to a *difference* in temperature is a continuous discharge of action potentials and there is additional evidence that this is so (Bullock & Cowles, 1951; Zotterman, 1953). It is thus tempting to conclude that free nerve endings are potentially capable of responding in a characteristic way and, by virtue of their three-dimensional position in the skin, in a characteristic pattern; and, because of overlap, in a unique spatio-temporal pattern as the result of the application of different stimuli. The force of this contention is augmented as the result of the observations in the preceding papers, and also in view of the fact that in the skin of all vertebrates lower than the amphibians which are subserved by relatively few dorsal root axons, only unencapsulated (free) nerve endings can be demonstrated and yet they respond to tactile, thermal (heat exchange) and other stimuli in a characteristic manner.

#### SUMMARY

1. At the base of the rabbit ear the great auricular nerve contains between 18 and 22 fasciculi and the lesser occipital nerve between 12 and 15 fasciculi. Together they contain approximately 6000 dorsal root myelinated axons.

2. Each fasciculus entering the base of the ear contains a varying number of myelinated axons, the diameters of which are representative of those appearing in the named nerves (i.e. great auricular and lesser occipital) as a whole. An interchange of axons is continually taking place between the fasciculi of the named nerves throughout their course to a greater or lesser degree.

3. Each fasciculus entering the base of the ear eventually terminates in the cutaneous plexus in which the axons divide and subdivide a large number of times. About three-quarters of the myelinated axons of all diameters in the named nerves terminate in relation to hair follicles.

4. Axons in fasciculi at the base of the ear subserve widely differing areas of skin, some of which are in the front of the ear. There is no linear relationship between the number of axons in a given fasciculus and the area of skin which it subserves.

5. The smallest hair follicles have at least two stem fibres associated with them. Large hair follicles have between 6 and 10 and the largest between 20 and 30 stem fibres which give rise to separate groups of terminals related to each follicle.

6. In unoperated specimens of rabbit ear skin, there were only 1 in 2000 degenerating nerve fibres seen in fasciculi throughout the whole extent of the back of the ear. No degenerating axons were seen in the cutaneous plexus or leaving the plexus on their way to hair follicles.

7. Following the excision of a single fasciculus at the base of the ear, there is a relatively circumscribed area in which the proportion of degenerating axons is highest. Nevertheless, a few degenerating axons related to hair follicles of all sizes are seen throughout the dorsum of the ear after the excision of a single fasciculus at the base of the ear.

8. Following the excision of fasciculi at the level of the vertebral column, a number of degenerating axons were seen in every fasciculus of the named nerve at the base of the ear and to degenerating nerve fibres in the cutaneous nerve plexus throughout the dorsum of the ear.

9. The greater part of the skin over the ventral surface of the ear is supplied by the great auricular and lesser occipital nerves. Fasciculi which contain axons subserving skin at the margin of the ear continue around the margin to terminate in the cutaneous plexus and so to supply hairs on the ventral surface of the ear.

10. Each fasciculus dissected free from the great auricular and lesser occipital nerves at the base of the ear subserves an area of skin on the dorsum of the ear which can be clearly defined by recording action potentials by brushing the hair stumps.

11. The areas subserved by 18 fasciculi in a single ear were outlined. A small zone of skin was found to be subserved by six dorsal root axons.

12. The areas subserved by 67 fasciculi in twelve rabbits at the base of the ear were mapped, measured and scaled for comparison with an ear having a dorsal skin surface area of 31 cm.<sup>2</sup> (average ear). The sum of the total areas subserved by the 'average' number of fasciculi entering an ear was found to be four times greater than the area of the skin covering the dorsum of an ear. Thus, if each hair or group of hairs emerging from a single orifice were to receive an equal number of dorsal root axons, each would receive four. It has been shown that in some areas single hairs or groups of hairs emerging from a single orifice are subserved by more than four dorsal root axons. It therefore follows that other hairs must be subserved by less than four axons.

13. Large action potential spikes of simple wave form were recorded from fasciculi near the tip of the ear by stimulation of hair stumps at the centre of the active area; smaller spikes were evoked towards the periphery.

14. Large, small and complex action potential spikes were recorded from fasciculi at the base of the ear by stimulation of hair stumps at both the centre and at the periphery of the active area.

15. Between 57 and 100 % of the hair stumps in areas subserved by fasciculi at the base of the ear give rise to action potentials when individually stimulated.

16. Fasciculi, sampled close to the vertebral column and containing an equivalent number of axons to those entering the base of the ear subserve areas amounting to

about half the dorsal surface of the ear. This indicates that a major regrouping of axons has taken place between the base of the ear and the vertebral column.

17. Areas subserved by fasciculi extending between the dorsal root ganglion and the spinal cord of equivalent perimeter to those sampled at the level of the vertebral column approximated in size to the areas encountered when recording from fasciculi of equivalent size at the base of the ear. This indicates that a second major regrouping of the axons takes place in the region of the dorsal root ganglia.

18. The bearing of these observations on the mechanism of cutaneous sensibility in man has been discussed.

This work was made possible by a grant from the Rockefeller Foundation which is gratefully acknowledged. We were also helped by the valuable suggestions of Dr W. Pallie, Mrs Elizabeth Palmer and Dr P. P. Lele. We would also like to thank Miss Christine Court, Miss Jean Gurden and Mr Frank Blackwell for their skilled technical assistance.

#### APPENDIX

The validity of the all-or-nothing law permits the use of the term binary system in reference to a single nerve fibre considered as an information system. A binary system can only convey one unit of information ( $\log_2 2 = 1$ ). In other words, an axon considered as a binary system is capable of transmitting only one unit of information, i.e. whether the hair to which (in the classical theory) it is exclusively attached has been stimulated or not.

However, it has been demonstrated that an individual myelinated axon innervates on the average eighty hairs. Thus, an individual action potential has a probability of  $1/80$  of having come from any given hair in the region of skin innervated by the axon. Since, according to Goldman (1953):

$$\text{information} = \log_2 1/p = \log_2 80 = 6.32 \text{ units of information,}$$

where

$$p = \text{probability} = 1/80 \text{ of a given hair being stimulated,}$$

there must be 6.32 binary units or binary systems *if* the hair is to be localized. The table which follows shows the relationship between the number of hairs innervated by each axon and the number of binary systems required for localization (assuming that each axon subserves the same number of hairs).

It follows, therefore, that the number of axons necessary for the localization of a hair subserved by an axon which innervates a total of 80 hairs differs by two

The number of hairs innervated by each axon	Binary systems required for localization	The number of hairs innervated by each axon	Binary systems required for localization
1	0	20	4.32
2	1	30	4.90
3	1.58	40	5.32
4	2	50	5.64
5	2.32	60	5.90
6	2.58	70	6.13
7	2.80	80	6.32
8	3.0	90	6.49
9	3.17	100	6.64
10	3.32	110	6.78
		120	6.90



integral units only from the number necessary to localize a hair which is innervated by an axon subserving a total of 120 hairs. For this reason, a hair innervated by six separate axons could be localized with a fair degree of accuracy whether the axon supplying it innervated anywhere between 30 and 120 hairs.

## REFERENCES

- ADEY, W. R., CARTER, I. D. & PORTER, R. (1954). Temporal dispersion in cortical response. *J. Neurophysiol.* **17**, 167–182.
- ADRIAN, E. D. (1931). Croonian Lecture: 'The messages in sensory nerve fibres and their interpretation'. *Proc. Roy. Soc. B*, **109**, 1–18.
- ALVAREZ-BUYLLA & DE ARELLANO, R. (1953). Local responses in Pacinian corpuscles. *Amer. J. Physiol.* **172**, 237–244.
- BISHOP, G. H. (1943). Responses to electrical stimulation of single sensory units of skin. *J. Neurophysiol.* **6**, 361–382.
- BULLOCK, T. H. & COWLES, R. B. (1952). Physiology of an infrared receptor: the facial pit of pit vipers. *Science*, **115**, 541–543.
- COLLIN, R. (1949). An instrument for the stimulation of single hairs. *J. Physiol.* **110**, 7–8.
- FULTON, J. F. (1943). *Physiology of the Nervous System*, p. 6. Oxford Medical Publications: 2nd ed. Oxford University Press.
- GOLDMAN, S. (1953). *Information Theory*, p. 5. London: Constable and Co. Ltd.
- GRAY, J. A. B. & SATO, M. (1953). Potentials from a Pacinian corpuscle. *J. Physiol.* **122**, 27.
- HAGEN, E., KNOCH, H., SINCLAIR, D. C. & WEDDELL, G. (1953). The role of specialized nerve terminals in cutaneous sensibility. *Proc. Roy. Soc. B*, **141**, 279–287.
- KATZ, B. & SCHMITT, O. H. (1940). Electric interaction between two adjacent nerve fibres. *J. Physiol.* **97**, 471–488.
- KUHN, R. A. (1953). Organization of tactile dermatomes in cat and monkey. *J. Neurophysiol.* **16**, 169–182.
- LELE, P. P. (1954). Relationship between cutaneous thermal thresholds, skin temperature and cross-sectional area of the stimulus. *J. Physiol.* **126**, 191–205.
- LELE, P. P., WEDDELL, G. & WILLIAMS, C. M. (1954). The relationship between heat transfer, skin temperature and cutaneous sensibility. *J. Physiol.* **126**, 206–234.
- MÜLLER, J. (1842). *Elements of Physiology*, **2**, 1059–1087. London: Taylor and Walton.
- SINCLAIR, D. C., WEDDELL, G. & ZANDER, E. (1952). The relationship of cutaneous sensibility to the neurohistology in the human pinna. *J. Anat., Lond.*, **86**, 402–411.
- TOWER, S. S. (1940). Units for sensory reception in cornea. *J. Neurophysiol.* **3**, 486–500.
- TYRRELL, H. J. V., TAYLOR, D. A. & WILLIAMS, C. M. (1955). Free nerve endings as transducers of thermal stimuli. *Nature, Lond.*, **174**, 918–921.
- WALSHE, F. M. R. (1942). The anatomy and physiology of cutaneous sensibility: a critical review. *Brain*, **65**, 48–112.
- WATERSTON, D. (1923). The sensory activities of the skin for touch and temperature. *Brain*, **46**, 200–208.
- WEDDELL, G. (1941). The pattern of cutaneous innervation in relation to cutaneous sensibility. *J. Anat., Lond.*, **75**, 346–367.
- WEDDELL, G. (1953). 'Cutaneous sensibility.' A chapter published in *Modern Trends in Dermatology*, 2nd Series, ed. R. M. B. MacKenna. London: Butterworth and Co. Ltd.
- WEDDELL, G. & GLEES, P. (1941). The early stages in the degeneration of cutaneous nerve fibres. *J. Anat., Lond.*, **76**, 65–93.
- WEDDELL, G. & PALLIE, W. (1954). The value of 'spreading factors' in the demonstration of tissue neural elements. *Quart. J. micr. Sci.* **95**, 389–397.
- WEDDELL, G., PALLIE, W. & PALMER, ELIZABETH (1954). The morphology of peripheral nerve terminations in the skin. *Quart. J. micr. Sci.* **95**, 483–501.
- WEDDELL, G., PALMER, ELIZABETH & PALLIE, W. (1955). Nerve endings in mammalian skin. *Biol. Rev.* (in the Press).
- ZOTTERMAN, Y. (1939). Touch, pain and tickling: an electrophysiological investigation on cutaneous nerves. *J. Physiol.* **95**, 1–28.
- ZOTTERMAN, Y. (1953). Special senses: thermal receptors. *Ann. Rev. Physiol.* **15**, 357–372.

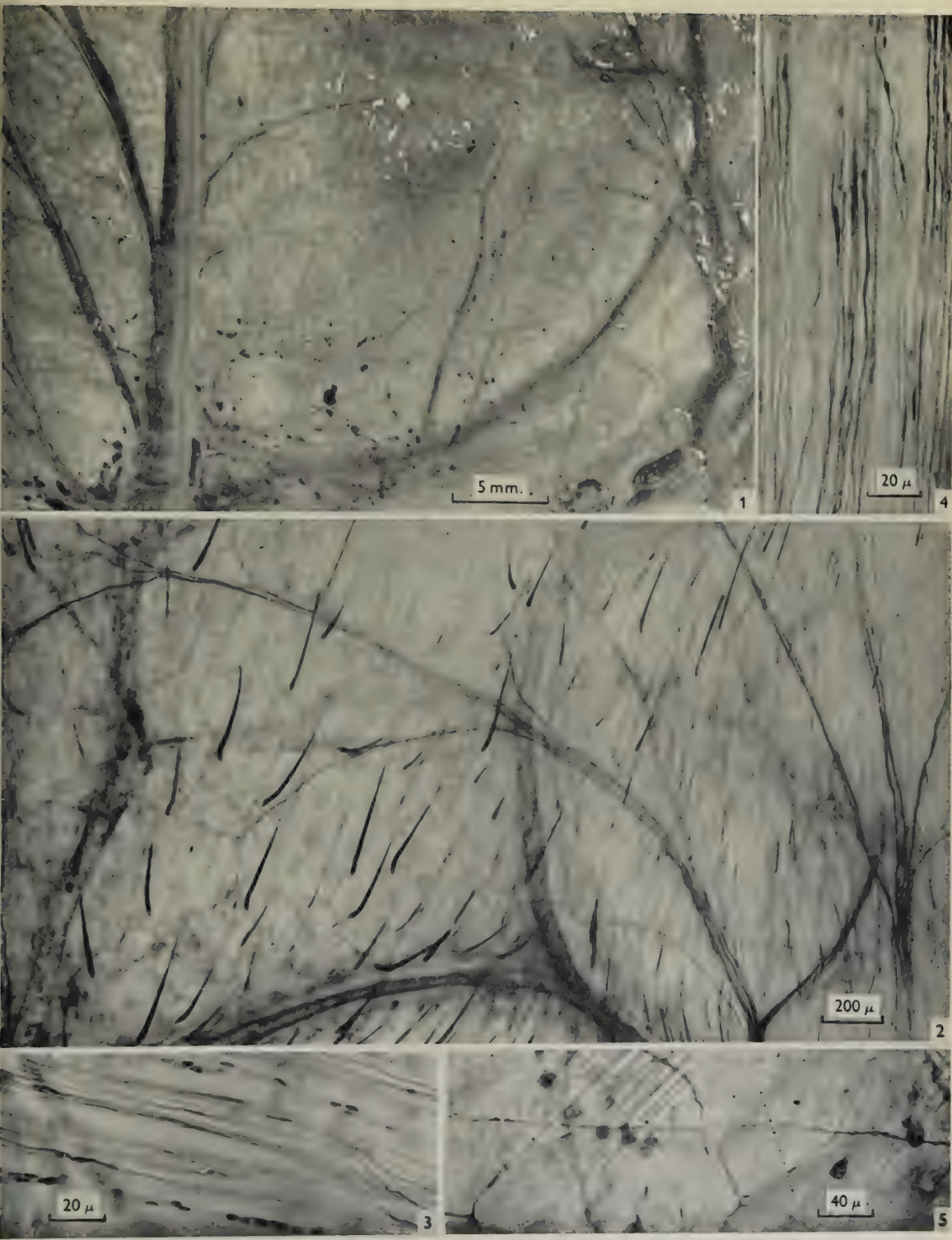
## EXPLANATION OF PLATES

## PLATE 1

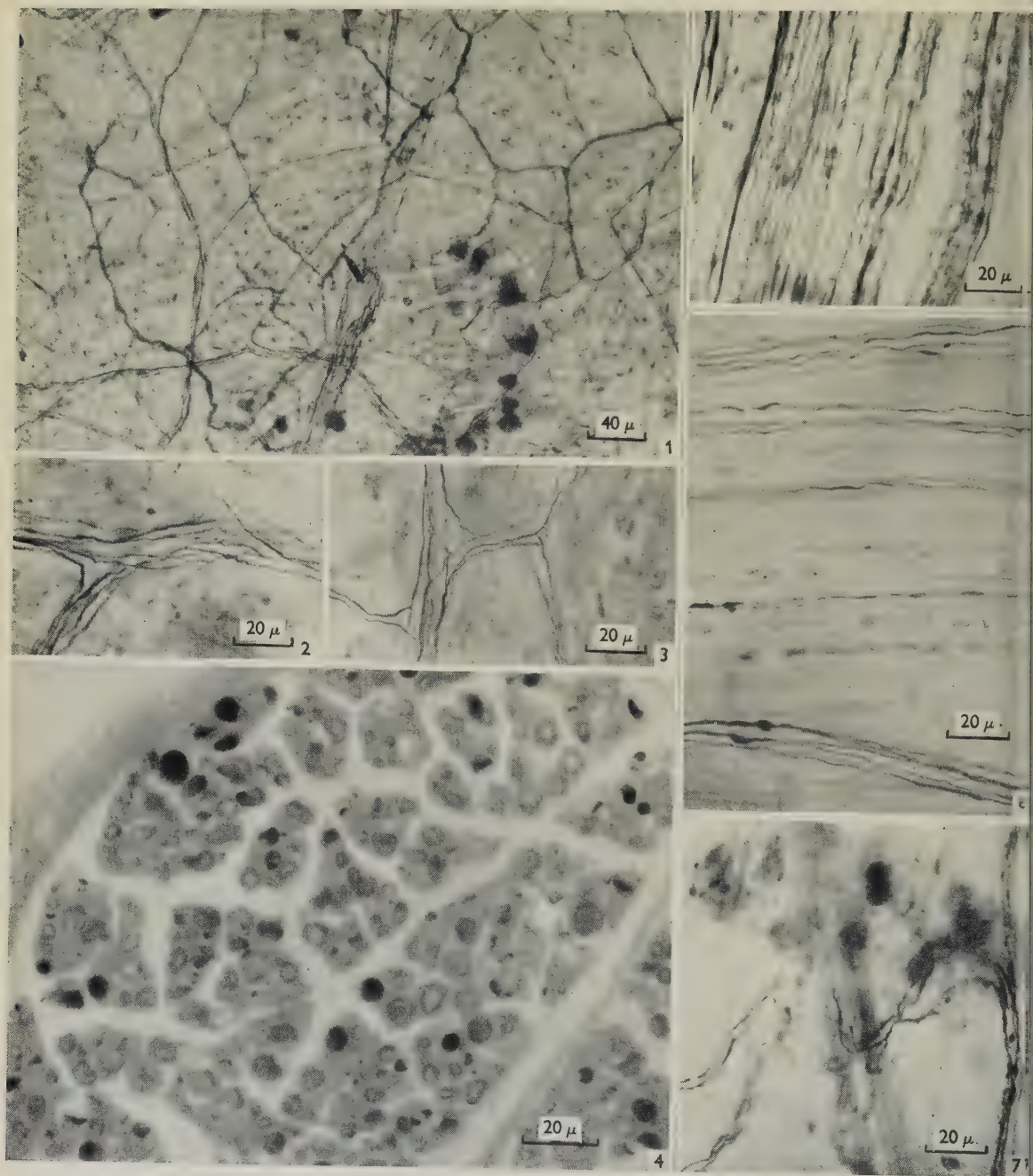
- Fig. 1. The great auricular (left) and lesser occipital nerves (right) entering the base of the distal three-quarters of the rabbit ear. The named nerves are seen to be in the form of flattened bands of contiguous fasciculi. Osmium tetroxide preparation.
- Fig. 2. Fasciculi leaving the lesser occipital nerve and scattering in all directions before terminating in the cutaneous plexus. One fasciculus is giving rise to branches which proceed for some distances towards the base of the ear before terminating. Osmium tetroxide preparation.
- Fig. 3. A fasciculus in the great auricular nerve near the base of the ear dividing relatively abruptly into two fasciculi. Methylene-blue preparation.
- Fig. 4. An interchange of axons between fasciculi in the great auricular nerve close to the base of the ear. Methylene-blue preparation.
- Fig. 5. An isolated myelinated axon pursuing a course independent of the cutaneous plexus in the dermis of a rabbit ear. Methylene-blue preparation.

## PLATE 2

- Fig. 1. Axons leaving a fasciculus of the great auricular nerve to enter the cutaneous plexus. Note the widespread scattering of the axons and the pattern in which they are arranged. Methylene-blue preparation.
- Figs. 2 and 3. The manner in which axons in the cutaneous plexus terminating in hair follicles (and elsewhere) scatter and multiply. Methylene-blue preparation.
- Fig. 4. Degenerating myelinated axons of all sizes scattered in a random manner throughout a fasciculus of the great auricular nerve at the base of the ear (all the other fasciculi were similarly affected) after excision of a single small fasciculus from the nerve at the level of the vertebral column. Marchi preparation.
- Fig. 5. Normal and degenerating axons lying side by side in a fasciculus of the great auricular nerve, one centimetre from the point at which a single small fasciculus of comparable size had been excised at the base of the ear. Methylene-blue preparation.
- Fig. 6. A few degenerating axons among normal axons in a fasciculus of the lesser occipital nerve towards the tip of the ear following excision of a single small fasciculus from the great auricular nerve at the base of the ear. Methylene-blue preparation.
- Fig. 7. Two degenerating axons lying among normal axons. One of the degenerating axons ends in relation to a hair follicle which also receives normal axons. The specimen, which was removed from the tip of the ear, lay three centimetres distant from the area of maximal degeneration seen histologically. It lay approximately the same distance from the area outlined by brushing hair stumps before the single fasciculus was excised from the great auricular nerve at the base of the ear. Methylene-blue preparation.







# THE SURFACE FEATURES OF THE BRAIN OF THE HUMPBAC WHALE (*MEGAPTERA NOVAEANGLIAE*)

By A. S. BREATHNACH

*Department of Anatomy, St Mary's Hospital Medical School, London*

## INTRODUCTION

Being highly adapted to an aquatic mode of life, the Cetacea present an interesting study both from a morphological and a functional point of view. This applies especially to the brain in these animals as owing to the particular environment in which they have undergone their development, some of the factors responsible for the form and proportions of the brain in living members of the group must have been very different from those operating in the majority of terrestrial mammals.

There are many gaps in our knowledge of the cetacean brain largely because of the difficulty of obtaining specimens in a suitable state for histological examination. As far as can be determined, the literature dealing with the brain of the humpback whale consists of two illustrations of Eschricht's (1869) and a brief account of a foetal brain by Guldberg (1885), together with one or two papers by Riese (1928, 1936) dealing with certain aspects of its development.

Accordingly, as some time ago the brain of an adult humpback whale in a reasonable state of preservation became available it was felt that a brief description might be of value. The present account is confined to the gross features and is largely given from the point of view of comparison with previous accounts of other cetacean brains. It is hoped in the future to give some account of the microscopic features of those parts which prove to be in a suitable state for histological examination.

Specimens of the brain of the fin-whale (*Balaenoptera physalis*), common porpoise (*Phocaena phocaena*) and of the bottle-nose dolphin (*Tursiops truncatus*) were available for comparison.

## GENERAL FORM AND DIMENSIONS

As may be seen from the figures (Text-fig. 1, Pl. 1, figs. 1, 2), the general form and proportions of the various parts of the brain conform to the typical cetacean pattern. One notes the marked brachyencephaly, the exuberant folding of the cerebral cortex, the almost vertical disposition of the Sylvian fissure with its surrounding series of concentric sulci, and the large size of the cerebellum.

The two halves of the brain are reasonably symmetrical, except for the cerebellum, the right half of which appears to be somewhat smaller than the left. However, each half weighs approximately the same, and this applies as well to the two halves of the entire brain. It is probable that in brains of this size minor degrees of asymmetry such as have been noted by previous authors, e.g. Guldberg (1885) may depend upon the position in which they happened to be placed during fixation, although this does not appear to be entirely the case where the odontocete brain is concerned (cf. Kojima, 1951).

Certain dimensions of the brain are given below. Unfortunately, nothing is known of the body dimensions of the individual (a female) from which the specimen was removed.

*Weight*

Entire brain without dura	4030 g.
Cerebellum	740 g.
Cerebellar percentage of total	18 %

*Linear*

Antero-posterior diameter of cerebral hemisphere	18.0 cm.
Dorso-ventral diameter of cerebral hemisphere	15.0 cm.
Transverse diameter of cerebral hemisphere	12.0 cm.
Transverse diameter of cerebellum	15.0 cm.
Cranio-caudal diameter of pons	3.5 cm.
Transverse diameter of pons (between fifth nerves)	3.5 cm.

#### VENTRAL ASPECT OF THE BRAIN AND ORIGIN OF THE CRANIAL NERVES

This is illustrated in Text-fig. 1, and since the arrangement is practically identical with that found in other cetaceans, little comment is necessary.

The olfactory peduncle measured 18 cm. in length, was 4 mm. wide and 1.5 mm. thick. As such it is somewhat wider and thicker than the human olfactory peduncle ( $3 \times 1$  mm.) and on section was seen to be composed of numerous fine nerve fibres. The olfactory bulb was not present, so presumably the peduncle was longer than the figure quoted by an unknown amount. Macroscopic medial and lateral olfactory tracts were not observed, but the diagonal band was very evident, a characteristic feature of the cetacean brain (Breathnach, 1953). Distinct mamillary bodies could not be identified.

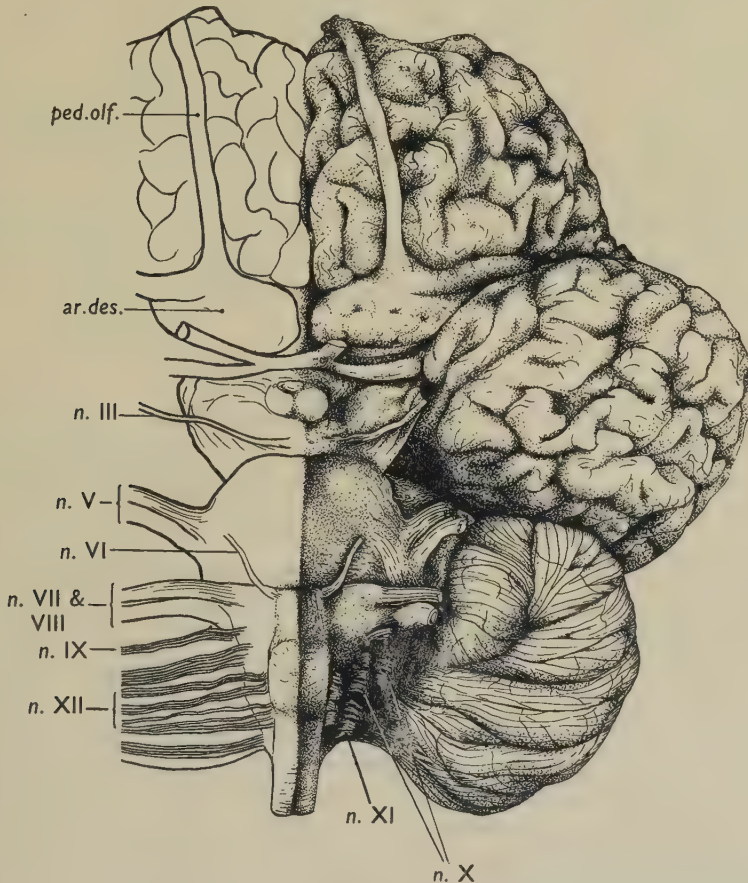
The fifth is the largest of the cranial nerves and springs from the pons in a manner similar to that in the fin-whale (Jansen, 1953). The proximal portion is filamentous, the more distal and larger part forming a solid trunk. A prominent obliquely directed bundle of pontine fibres descends between the origin of this nerve and that of the seventh, and may correspond to the oblique bundle of the pons of human anatomy.

The eighth nerve comes next in size to the fifth. It is interesting to note that there appears to be a distinct difference between the two groups of Cetacea so far as the relative macroscopic sizes of these two nerves are concerned. In the Mysticeti (present account; Wilson, 1933; Jansen, 1953) the fifth is the larger, whereas in the Odontoceti (Langworthy, 1932; Ries & Langworthy, 1937) the reverse is the case, and the acoustic centres in the mid-brain are relatively larger.

The ninth nerve is slender and arises by a series of obliquely placed rootlets caudal and medial to the origin of the eighth (Text-fig. 1). The tenth and eleventh nerves (Text-fig. 1; Pl. 2, figs. 3, 4) arise in a manner similar to that figured by Jansen (1953) in the fin-whale. The tenth is comprised of two series of rootlets, medial and lateral which may as suggested by Jansen correspond to the motor and sensory roots respectively of the vagus. The bulbar portion of the accessory nerve



is in direct linear continuity caudally with the medial rootlets of the vagus, and the spinal portion (Pl. 2, fig. 3) springs from the lateral aspect of the spinal medulla opposite the first two cervical segments. The twelfth nerve arises by a series of ten to twelve rootlets lateral to the olivary eminence, which in cetacea is formed by the prominent medial (accessory) olive. The pyramids are small and cannot be seen below the level of the upper border of the olive over the surface of which the fibres are spread.



Text-fig. 1. A drawing of the ventral aspect of the brain to show the origins of the cranial nerves.  $\times 0.5$ .

#### MEDIAN SAGITTAL SECTION OF BRAIN STEM

This is figured in Pl. 1, fig. 2. The corpus callosum is of moderate size and has an orientation typical for cetaceans. There is no rostrum and the cavum septi is open as in the porpoise (Breathnach, 1953). The margin of the fornix towards the septum pellucidum is difficult to define, and in this situation it forms a much less compact bundle than, for instance, in the porpoise.

The depth of the third ventricle greatly exceeds the antero-posterior diameter,

a characteristic cetacean feature which can be associated with the shape of the brain as a whole. An ill-defined small anterior commissure could be observed half-way up the lamina terminalis, but it does not show particularly well in the figure. Well-marked preoptic and infundibular recesses are present in the floor of the ventricle, which is particularly thin in the mid-line of the tuberal region. The inter-thalamic connexus is extensive and accentuates the well-marked hypothalamic sulcus which lies ventral to it. The pineal could not be identified and may have been removed with the membranes. In this regard it is of interest to note that Fuse (1936) found the pineal to be rudimentary in the series of cetaceans examined by him, whereas Gersch (1938) had no difficulty in identifying it in the humpback.

The aqueduct is voluminous, another typical cetacean feature (Ries & Langworthy, 1937). A considerable portion of the lateral wall is formed by the tectum, and a small recess extends laterally between the posterior aspect of the inferior colliculus and the superior medullary velum. (This is accentuated somewhat in the photograph by a slight detachment of the velum in this situation.) The velum is extensive and can be followed posteriorly as far as the apex of the median dorsal recess of the IVth ventricle. The cerebellum is described later (p. 348).

#### CEREBRAL HEMISPHERES

A markedly convoluted surface with the fissures on the lateral aspect arranged in an arcuate manner around the Sylvian fissure is characteristic of the cetacean cerebral hemisphere. The temporal lobe is markedly developed. These features can be seen in Text-fig. 1, and Pl. 1, figs. 1 and 2.

Previous authors (Guldberg, 1885; Rawitz, 1910; Kojima, 1951, etc.) have been much concerned about the comparative homology of the cerebral fissures of cetaceans, but their labours have produced little result beyond varying degrees of disagreement over the naming of particular fissures, largely because of a lack of criteria suitable for their identification. In the case of certain fissures, e.g. the rhinal, which separate cortical areas of widely differing structure, histological examination may help in identification (Breathnach, 1953), and in the case of others such as the sulcus cinguli, topographical relations may help. As regards the majority of fissures on the supero-lateral surface, however, one can merely speculate, and as this is likely to be a particularly barren exercise, no attempt is made in the present account to enter into this matter.

A well-marked Sylvian fissure is present (Pl. 1, fig. 1) at the bottom of which an extensive area of operculated cortex is found covering the lateral aspect of the corpus striatum. Following the practice of other authors (e.g. Guldberg, 1885) this may be called the 'insula'. Topographically it certainly corresponds with the insula of human and other primate brains, but whether the correspondence extends to cytoarchitectonic features such as those used by Rose (1928) to define 'insular cortex' it is not yet possible to say. In the humpback whale the Sylvian fissure is almost completely closed; in *Balaenoptera sibbaldi* Beaufort (1883) found it widely open, and Wilson (1933) described the insula as being almost entirely exposed in *Balaenoptera sulphurea*. Comparison between the humpback and fin-whale brains in our possession shows that the fissure is more widely open in the latter. From a

study of the literature it would appear that in spite of considerable variation there is a general tendency for the fissure to be more open in the Mysticeti than in the Odontoceti.

#### MESENCEPHALON

The cerebral peduncle is short and wide (Text-fig. 1), and a number of the surface fibres take an oblique course. A well-marked, narrow transverse peduncular tract (Pl. 2, fig. 4) can be traced along the lateral aspect from the neighbourhood of the upper edge of the superior colliculus to a point somewhat proximal and lateral to the origin of the third nerve. This tract has also been noted by Wilson (1933) in *Balaenoptera* and by Kojima (1951) in *Physeter*.

The tectum is large and forms not only the roof but a considerable portion of the side wall of the large aqueduct. There is a marked difference in shape between the two colliculi, and because of this, it is very difficult to give an accurate estimate of their relative sizes. Viewed from the dorsal (Pl. 2, fig. 3) and medial (Pl. 1, fig. 2) aspects, the superior one appears to present the greater surface area, while on lateral view the inferior is by far the larger (Pl. 2, fig. 4). The overall impression gained by macroscopic examination is that the inferior colliculus has the larger volume, although a definite answer can only be reached on microscopic examination. Guldberg (1885), in his account of the brain of a foetal humpback, described the inferior colliculus as being the smaller. According to Wilson (1933) the inferior colliculus is larger in *Balaenoptera sulfurea* (although it is difficult to obtain an adequate impression from his figures), and Langworthy (1935) found the same to be the case in *B. physalis*. In the Odontoceti (e.g. *Tursiops* and *Physeter*), however, as pointed out by Langworthy, the reverse relationship holds, the inferior being the larger. It is possible that this apparent difference between the two groups may be correlated with the fact that the eighth nerve appears to be larger in the Odontoceti. However, estimates of size based upon surface appearances may be misleading, and one must be cautious of drawing functional conclusions from evidence of this character.

A broad mass of fibres connects the colliculi of opposite sides across the mid-line, and it is not easy to distinguish a distinct posterior commissure. The brachium of the inferior colliculus is easily recognized, and the lateral lemniscus forms a stout band as it emerges from the pons to pass backwards deep to the inferior colliculus (Pl. 2, fig. 4). The slender fourth nerve springs from the angle formed between the lateral lemniscus and the superior cerebellar peduncle a considerable distance from the mid-line. The medial geniculate body is prominent (Pl. 2, fig. 4), but the lateral geniculate produces no recognizable surface elevation.

#### MEDULLA AND PONS

The salient features of the ventral aspect have been described already and are illustrated in Text-fig. 1.

The cavity of the fourth ventricle is voluminous (Pl. 1, fig. 2), but remarkably little detail can be distinguished in the floor. There is no sign of striae medullares, and neither hypoglossal nor facial eminences can be defined (Pl. 2, fig. 3). The longitudinal bundle of fibres labelled 'medial longitudinal bundle' by Langworthy



(1932) in the porpoise, and also noted by Jansen (1953) in the fin whale, can be seen faintly. This absence of surface features which is in contrast to the findings of the above authors in the animals named, does not appear to be due to poor preservation of the material. A corresponding lack of detail was noted by Wilson (1933) in *Balaenoptera*.

#### CEREBELLUM

The cetacean cerebellum has attracted considerable attention in the past, partly on account of its large size. Amongst those who have previously attempted an analysis of the folial pattern may be mentioned Guldberg (1885), Bolk (1906) and Ogawa (1935). Unfortunately, most of these accounts are unsatisfactory largely because of differences in the terminologies used, but also because little or no embryological material was available with which to check the validity of the interpretation of the condition in the adult.

By far the most satisfactory account of the cetacean cerebellum is contained in the recent work of Jansen (1953, 1954) which is based upon a unique collection of graded foetal as well as adult material from the fin-whale. His identification of the various subdivisions differed so much from that of previous workers that it was considered of value to determine how closely his scheme can be applied to the cerebellum of the humpback whale.

Jansen adopts the usual subdivision into anterior and posterior lobes (comprising the corpus cerebelli) and a flocculo-nodular lobe. A similar subdivision can be used for the humpback whale; the fissura prima, separating anterior and posterior lobes can be readily identified in median section (Text-fig. 2) and can be followed into the hemispheres of the cerebellum (Text-fig. 3). The postero-lateral fissure can also be seen in median section (Text-fig. 2) where it separates the small nodule of the flocculo-nodular lobe from the uvula of the posterior lobe; its lateral extension will be described later.

##### *The anterior lobe (Text-figs. 2, 3 and 5)*

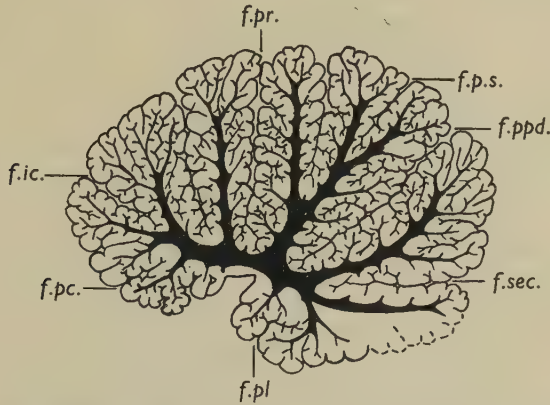
In median section (Text-fig. 2) the vermis of the anterior lobe in the humpback whale is practically identical with that of the fin-whale (see Jansen, 1954, fig. 20), and comprises about one-third of the area of the arbor vitae. Jansen's praeculminate and intraculminate fissures can easily be identified.

The hemispherical parts of the anterior lobe (Text-fig. 3) are small, as is generally the case in Cetacea (Jansen, 1953); that they appear somewhat larger in the humpback whale than in Jansen's illustration of the fin-whale is mainly due to a slight difference in the orientation of the specimens from which the figures were drawn.

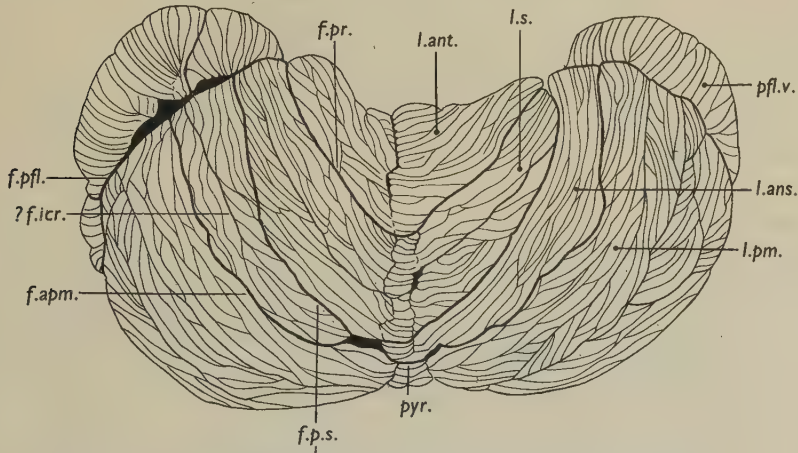
##### *The posterior lobe (Text-figs. 2-5)*

In median section (Text-fig. 2) the similarity to the fin-whale is not quite so marked as in the anterior lobe. There seems little doubt about the identification of the postero-lateral fissure, the fissura secunda and the praepyramidal fissure, defining the uvula and the pyramid of the vermis. These indeed resemble the corresponding parts in the fin-whale very closely. Cranial to the praepyramidal fissure, however, Jansen (1954) identifies two fissures in the fin-whale, the intercrural and the postero-superior fissures respectively. The postero-superior fissure, traced

laterally (Text-fig. 3), forms the posterior boundary of the lobulus simplex and on that account can be identified in the humpback whale and has been marked on the median section (Text-fig. 2). It is not so deep as in the fin-whale. The intercrural fissure, following Jansen, should be between the postero-superior and the praepyramidal fissures and can be traced (in the fin-whale) laterally into



Text-fig. 2. Mid-sagittal section of cerebellum. Drawn from a photograph.  $\times 0.8$ .



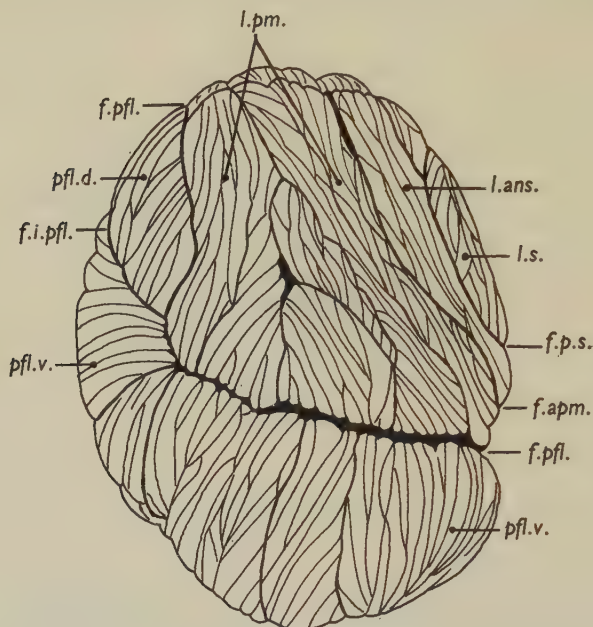
Text-fig. 3. The fissures and lobules on the dorsal aspect of the cerebellum. Drawn from a photograph.  $\times 0.5$ .

the ansiform lobule where it separates Crus I from Crus II. Apart from the very shallow indentation seen in median section in the humpback whale (Text-fig. 2, unlabelled) no such fissure is present in the vermis in this animal.

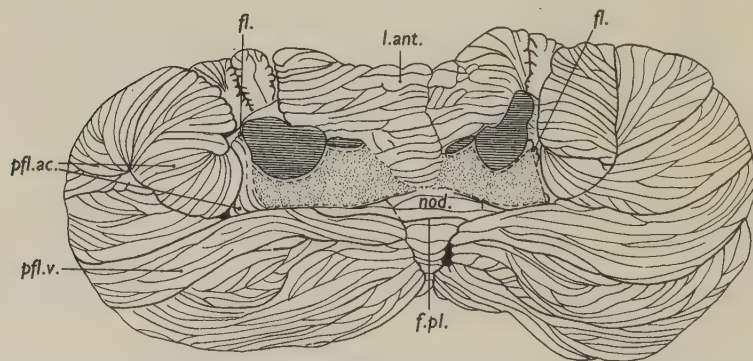
The hemispherical parts of the posterior lobe are the lobulus simplex, the ansiform lobule, the paramedian lobule and the paraflocculus.

The lobulus simplex forms the most cranial subdivision and lies between the fissura prima and the postero-superior fissure. It corresponds fairly closely in relative size and extent with Jansen's (1954) concept of this lobule in the fin-whale.

The ansiform lobule lies immediately behind the postero-superior fissure and is bounded caudally by the anso-paramedian fissure (Text-fig. 3). In the adult fin-whale Jansen (1954) described the anso-paramedian fissure as being apparently



Text-fig. 4. The fissures and lobules on the lateral aspect of the cerebellum. Drawn from a photograph.  $\times 0.8$ .



Text-fig. 5. Ventral view of cerebellum. Drawn from a photograph.  $\times 0.5$ .

continuous with the prepyramidal fissure on the surface, but stated that closer examination revealed several cortical lamellae which passed across the bottom of the praepyramidal fissure to connect the paramedian with the median part of the ansiform lobule (or tuber vermis). In effect, therefore, the anso-paramedian fissure swings on to the oral bank of the prepyramidal fissure. Since an exactly similar



arrangement is found in the humpback whale, there seems no doubt about the identification of the anso-paramedian fissure as marked in Text-fig. 3, and it should be noted that the ansiform lobule is considerably smaller than in the fin-whale. Moreover, as already pointed out, there is difficulty in defining an intercrural fissure in the humpback whale. It has been indicated tentatively on the left side of Text-fig. 3, but it does not reach the mid-line and nothing corresponding can be found on the right. While no doubt the cranial and caudal parts of the ansiform lobule represent Crus I and Crus II, as defined by Jansen in the fin-whale, it is clear that the subdivision between them is poorly indicated.

The paramedian lobule was defined by Jansen (1954) as 'bordered by the anso-paramedian and parafloccular fissures and medially continuous with the posterior tuber and the pyramis'. The portion of the cerebellar hemisphere in this position in the humpback whale is very similar in shape and extent with Jansen's paramedian lobule (Text-fig. 3). It forms nearly the whole of the lateral aspect of the cerebellum dorsal to the paraflocculus (Text-fig. 4) and is the second largest lobule of the cerebellum.

The paraflocculus forms the remainder of the posterior lobe between the fissura parafloccularis and the postero-lateral fissure. According to Jansen (1953) it is relatively enormous in Cetacea and he divides it into three parts, dorsalis, ventralis and accessorius.

The fissura parafloccularis can be readily identified in any cetacean cerebellum as the deep fissure which appears to divide the hemisphere into two main parts (Text-figs. 3, 4). Jansen (1953) defines the dorsal paraflocculus as an oval lobule lying between this fissure and the fissura intraparafloccularis. A lobule which is practically identical in appearance with the dorsal paraflocculus of Jansen's account is present in the humpback whale (Text-fig. 4). Medially it is continuous with the most rostral folia of the uvula.

The great size of the ventral paraflocculus, as well as the position of the accessory paraflocculus, may be seen from Text-figs. 4 and 5. These portions of the hemisphere have an extent and appearance closely similar to those of the same name figured by Jansen (1954) in the fin-whale. It should be noted that in this publication Jansen included in the accessory paraflocculus the part labelled *p. fl. v. x.* in his 1953 account in addition to the group of folia lying deep to it (his original accessory paraflocculus).

#### *Flocculo-nodular lobe (Text-figs. 2, 5)*

The position of the postero-lateral fissure on the median section can be seen from Text-fig. 2. On the ventral aspect the vermian portion of this fissure is clearly defined (Text-fig. 5) and when traced laterally, it appears to extend in such a manner as to necessitate including a part which obviously corresponds with the deeper part of the accessory paraflocculus of Jansen's (1953) account, within the flocculo-nodular lobe as part of the flocculus. Closer examination, supplemented by a study of the fin-whale material available (where the condition of the flocculo-nodular lobe is identical with that figured by Jansen) indicated that this was an erroneous interpretation due to the fact that the intermediate portion of the postero-lateral fissure is extremely shallow in places. This is a condition which is apparently quite common

in adult animals, cf. Larsell (1953), and may explain previous misinterpretations of the extent of the flocculus in cetacean brains.

The flocculus in the humpback consists of five to six poorly developed folia which partly cover over the roof of the lateral recess (Text-fig. 5) and are connected medially with the nodule by means of an attenuated peduncle composed of two or three very narrow folia. This condition is identical with that found by Jansen (1953) in the fin-whale.

From the above description it will be seen that Jansen's scheme of cerebellar subdivision fits the folial pattern as seen in the humpback whale very closely. The only large difference encountered was the small size of the ansiform lobule and the absence of a well-developed fissura intercruralis. This lobule seems to be particularly liable to variation in size and arrangement in different animals, and at present no great significance can be attached to its small size in the humpback whale.

#### DISCUSSION

It is clear that the brain of the humpback whale (*Megaptera novaeangliae*) does not differ markedly from those of other Mysticeti, and there are few matters which call for discussion so far as the gross morphology is concerned.

A feature of the cetacean brain to which attention has frequently been drawn is the reduction or absence of the olfactory apparatus, and it is of interest to note that the olfactory peduncle in the humpback is of considerable size and contains a considerable number of olfactory tract fibres. The condition of the olfactory mucosa, the primary receptor surface, is not known, but the central neurological part of the olfactory apparatus has certainly been preserved to a much greater extent than in the Odontoceti and would appear capable of functioning. Whether olfaction plays any important part in the life and behaviour of the animal is, however, extremely doubtful, since the blow-hole is closed except during the brief periods when the animal is on the surface. It seems more likely that the olfactory apparatus in the Mysticeti is comparable to the muscles of the human auricle, which, while preserving all the neurological connexions necessary for active function, play no significant part in human behaviour. The fact that the olfactory bulbs and peduncles have been completely lost in the Odontoceti supports this hypothesis.

It is commonly thought that olfactory connexions are of considerable importance in determining the morphology of the forebrain as a whole, and this is probably true in the early stages of the evolution of vertebrates. Once the basic pattern of the forebrain is established, however, the fate of the olfactory apparatus seems to be of very limited significance. This conclusion was reached by Armstrong, Gamble & Goldby (1953) as a result of their examination of the brain of *Anolis*, a microsmatic lizard, and is supported by the conditions in cetaceans. The differences between the brains of the Mysticeti and the Odontoceti can be attributed far more plausibly to the differences in the morphology of the skull, than to the presence of olfactory tracts in the one and their absence in the other. It is indeed probable that the loss of the olfactory bulbs and tracts in the Odontoceti is itself related to mechanical factors in the development of the skull, as suggested by Howell (1930). These must be very different from those obtaining in the Mysticeti, although the final result in the 'telescoping' of the cranial cavity is somewhat similar in both groups (Miller, 1923).

Langworthy (1931) has pointed out that the acoustic division of the eighth nerve is probably the paramount sensory cranial nerve in living cetaceans, and it is tempting to relate the greater development of the acoustic nerve and centres in the odontocete as compared with the mysticete brain with the lesser development of the olfactory connexions in the former. There is, however, no reason whatever for postulating a reciprocal relationship between these two nerves of special sense; either might rise to dominance, or become suppressed, quite independently of the other.

In the cerebellum it can be seen that the subdivisions recognized by Jansen (1953, 1954) in the fin-whale can all be readily identified in the humpback whale, and that the relative proportions of the different parts are almost exactly similar. While, in the absence of foetal material, the present study can give no information about the sequence of appearance of the fissures, the similarity in the adult stages makes it very probable that Jansen's findings in the fin-whale would be equally applicable to the humpback whale.

# SUMMARY

1. The surface appearances of the brain of the humpback whale are described. Apart from differences in size the arrangement does not differ appreciably from that of other mysticete brains which have been described.

2. Jansen's scheme of cerebellar subdivision fits closely to the pattern found in the humpback whale.

Thanks are due to Mr R. M. Brachi, and Messrs Hector Whaling Ltd., London, for collecting the specimen; to Prof. F. Goldby for advice and criticism; and to the Photographic Department, St Mary's Hospital Medical School, for the photographs which appear in Pls. 1 and 2. Text-fig. 1 was drawn by Miss Jill Payne.

# ABBREVIATIONS FOR ALL FIGURES

<i>ar.des.</i>	area désert	<i>hyp.</i>	hypophysis
<i>aq.cer.</i>	aqueductus cerebri	<i>l.ans.</i>	lobulus ansiformis
<i>br.pont.</i>	brachium pontis	<i>l.ant.</i>	lobus anterior
<i>c.cal.</i>	corpus callosum	<i>l.pm.</i>	lobulus paramedianus
<i>ch.</i>	chiasma opticum	<i>l.s.</i>	lobulus simplex
<i>col.inf.</i>	colliculus inferior	<i>lem.lat.</i>	lemniscus lateralis
<i>col.sup.</i>	colliculus superior	<i>n. II-XII</i>	cranial nerves
<i>cor.gen.med.</i>	corpus geniculatus medialis	<i>nod.</i>	nodulus
<i>f.apm.</i>	fissura anso-paramedianus	<i>ped.olf.</i>	pedunculus olfactorius
<i>f.i.pfl.</i>	fissura intra-parafloccularis	<i>pfl.ac.</i>	paraflocculus accessorius
<i>f.ic.</i>	fissura intraculminata	<i>pfl.d.</i>	paraflocculus dorsalis
<i>f.icr.</i>	fissura intercruialis	<i>pfl.v.</i>	paraflocculus ventralis
<i>f.p.s.</i>	fissura postero-superior	<i>pyr.</i>	pyramis cerebelli
<i>f.pc.</i>	fissura praeculminata	<i>str.t.</i>	stria terminalis
<i>f.pfl.</i>	fissura parafloccularis	<i>tr.opt.</i>	tractus opticus
<i>f.pl.</i>	fissura postero-lateralis	<i>tr.ped.</i>	tractus peduncularis transversus
<i>f.ppd.</i>	fissura praepyramidalis	<i>thal.</i>	thalamus
<i>f.pr.</i>	fissura prima	<i>v. IV</i>	ventriculus quartus
<i>f.sec.</i>	fissura secunda	<i>v.lat.inf.</i>	ventriculus lateralis (cornu inferior)
<i>f.syl.</i>	fissura sylvia	<i>v.med.ant.</i>	velum medullare anterior
<i>fl.</i>	flocculus		
<i>for.</i>	fornix		



## REFERENCES

- ARMSTRONG, J. A., GAMBLE, H. J. & GOLDBY, F. (1953). Observations on the olfactory apparatus and the telencephalon of *Anolis*, a microsmatic lizard. *J. Anat., Lond.*, **87**, 288-307.
- BEAUREGARD, H. (1883). Recherches sur l'encéphale des Balaenides. *J. Anat., Paris*, **19**, 481-516.
- BOLK, L. (1906). *Das Cerebellum der Säugetiere*. Haarlem: De Erven F. Bohn; Jena: Gustav Fischer.
- BREATHNACH, A. S. (1953). The olfactory tubercle, prepyriform cortex, and precommissural region of the porpoise (*Phocaena phocaena*). *J. Anat., Lond.*, **87**, 96-113.
- ESCHRIGHT, D. F. (1869). Ni Tavler til Oplysning av Hvaldyrenes Bygning. *K. danske vidensk. Selsk. Ser. 5*, **9**, 1-14.
- FUSE, G. (1936). Über die Epiphyse bei einigen wasserbewohnenden Säugetieren. *Arb. anat. Inst. Sendai*, **18**, 241-341.
- GERSCH, I. (1938). Note on the pineal gland of the humpback whale. *J. Mammal.* **19**, 477-480.
- GULDBERG, G. A. (1885). Über das Zentralnervensystem der Bartenwale. *Christiania Videnskabselskabs forhandl.* Pp. 1-154.
- HOWELL, A. B. (1930). *Aquatic Mammals*. Springfield, Ill: Charles G. Thomas.
- JANSEN, J. (1953). Studies on the cetacean brain. The gross anatomy of the rhombencephalon of the fin-whale (*Balaenoptera physalis*, L.). *Hvalråd. Skr.* **37**, 1-35.
- JANSEN, J. (1954). In *Aspects of Cerebellar Anatomy*. By Jansen, J. and Brodal, A. Oslo: Johan Grundt Tanum Forlag.
- KOJIMA, T. (1951). On the brain of the sperm whale (*Physeter catodon*, L.). *Sci. Rep. Whales Res. Inst., Tokyo*, **6**, 49-72.
- LANGWORTHY, O. R. (1931). Factors determining the differentiation of the cerebral cortex in sea-living mammals (the Cetacea). A study of the brain of the porpoise, *Tursiops truncatus*. *Brain*, **54**, 225-246.
- LANGWORTHY, O. R. (1932). A description of the central nervous system of the porpoise (*Tursiops truncatus*). *J. comp. Neurol.* **54**, 437-488.
- LANGWORTHY, O. R. (1935). The brain of the whalebone whale, *Balaenoptera physalis*. *Johns Hopkins Hosp. Bull.* **57**, 143-147.
- LARSELL, Ö. (1953). The cerebellum of the cat and the monkey. *J. comp. Neurol.* **99**, 135-200.
- MILLER, G. S. (1923). The telescoping of the cetacean skull. *Smithson. misc. Coll.* **76**, 1-71.
- OGAWA, T. (1935). Beiträge zur vergleichenden Anatomie des Zentralnervensystems der Wassersäugetiere. Über die Kleinhirnerkerne der Pinnipeden und Zetacien. *Arb. ant. Inst. Sendai*, **17**, 63-136.
- RAWITZ, B. (1910). Das Centralnervensystem der Cetacean. III. Die Furchen und Windungen des Gehirns von *Balaenoptera rostrata* Fabr. *Arch. mikr. Anat.* **75**, 225-239.
- RIES, F. A. & LANGWORTHY, O. R. (1937). A study of the surface structure of the brain of the whale (*Balaenoptera physalis* and *Physeter catodon*). *J. comp. Neurol.* **68**, 1-48.
- RIESE, W. (1928). Über das Vorderhirn des Wahlfoetus (*Megaptera Boops*). *Anat. Anz.* **65**, 255-260.
- RIESE, W. (1936). Über die Entwicklung des Wahlhirns. *Proc. Acad. Sci. Amst.* **39**, 97-109.
- ROSE, M. (1928). Die Inselrinde des Menschen und der Tiere. *J. Psychol. Neurol., Lpz.*, **37**, 467-624.
- WILSON, R. B. (1933). The anatomy of the brain of the whale (*Balaenoptera sulfurea*). *J. comp. Neurol.* **58**, 419-480.

## EXPLANATION OF PLATES

## PLATE 1

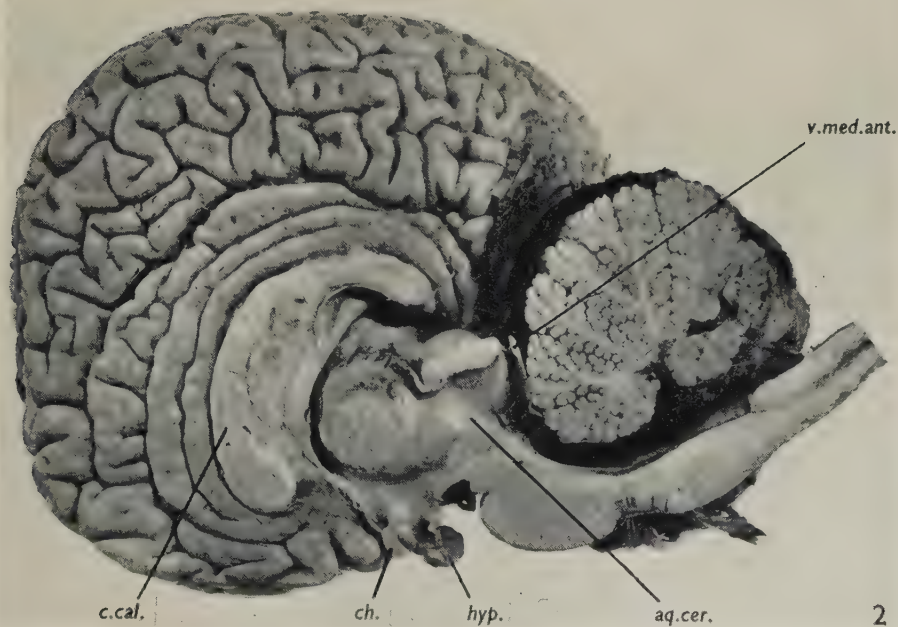
Figs. 1 and 2. Lateral and median sagittal aspects of the brain of *Megaptera novaeangliae*.  $\times 0.5$ .

## PLATE 2

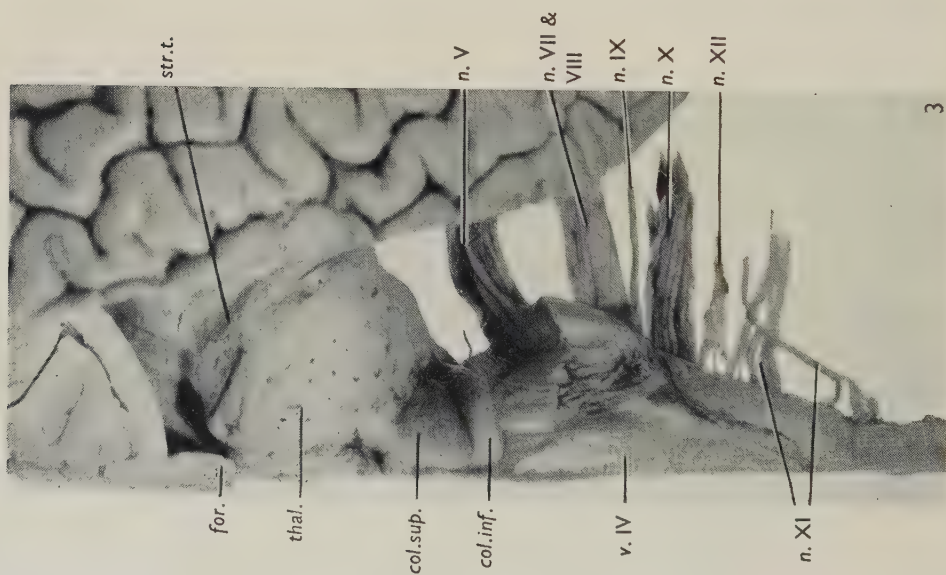
Figs. 3 and 4. Dorsal and lateral aspects of the brain stem of *Megaptera novaeangliae*.  $\times 0.6$ .



1



2





# THE POSITION OF THE HALLUX IN WEST AFRICANS

By N. A. BARNICOT

*Department of Anthropology, University College, London*

AND R. H. HARDY

*Department of Anatomy, University College, London*

## INTRODUCTION

Variation in the position of the great toe in adult Europeans remains a subject of discussion and conjecture, especially in relation to pathological degrees of valgus deformity. Operations to correct this deformity or its sequelae occupy a major place in orthopaedic operating lists, and methods of prevention are widely canvassed, particularly in connexion with the design of footwear.

Hardy & Clapham (1952) have shown that in school children the great toe has assumed a valgus position of adult degree by the age of 15 years, and they (1951) have also shown that females suffer a greater degree of deformity than males, and constitute 85% of those attending for subsequent orthopaedic correction. The associated widening of the angle between the first and second metatarsals has been shown by these workers to develop later than the valgus deviation of the toe and is not likely therefore to be a predisposing factor. Current opinion favours the effect of constricting footwear as a major cause of the deformity, and this view is supported by common-sense and observation alike (Craigmile, 1953). The importance of this factor is, however, difficult to assess in a habitually shod population, and for this reason it appeared useful to obtain some comparative data on the deviation of the great toe in a population which is barefooted.

A visit of one of us (N. A. B.) to Nigeria, West Africa, under a grant from the Colonial Medical Research Council, provided the opportunity to collect material from habitually barefooted Africans of both sexes and various ages.

## METHODS

Material was collected by taking footprints of the subjects with a Scholl Pedograph. This instrument consists essentially of a rubber membrane, inked on its lower surface, on which the subject places one foot and applies his whole weight, thereby making a print on a piece of cartridge paper placed below the membrane. The method is simple and rapid and provides a permanent record from which various measurements can later be obtained. It is clearly less valuable for our present purpose than are X-ray photographs, but X-ray equipment suitable for a field survey was not available. From previous experience with a European sample, it was found that the angle of the hallux measured on footprints in the manner indicated below showed a correlation ( $r$ ) of 0.56 with measurements of the angle between the 1st toe and metatarsal made on radiographs of the same sample; some caution is therefore required in arguing from data obtained by one method to that obtained by the other.

Measurements were taken on the footprints, which were all from the right foot, in the following way (see diagram, Fig. 1). A line was drawn from the most anterior point of the imprint of the 2nd toe (*A*) to the most posterior point of the heel (*B*); the length of this line was taken as the length of the foot. The most medial point in the region of the medial aspect of the 1st metatarso-phalangeal joint was then marked by eye (*C*), and a line was drawn from this point transecting the length line at right angles. A tangent parallel to the length line *AB* was drawn through the most laterally situated point on the lateral margin of the foot (*D*). The point (*E*) at which this tangent cut the transverse line was then found, and the line *CE* was taken as a measure of foot breadth. A line contacting the medial aspect of the heel imprint at (*F*) was drawn through the point *C* and further line contacting the medial aspect of the great toe at (*G*) was then drawn through *C* and prolonged to *H*. The angle *HCF* was taken as a measure of the hallux angle and was given a positive sign if the great toe deviated to the lateral side.

#### THE SAMPLE

The Nigerian sample was collected from towns and villages within 30 miles of Lagos. The majority of the subjects belonged to the Yoruba tribe.

With the exception of the series of Nigerian soldiers (kindly provided by Dr W. S. S. Ladell), who wore army boots, and of fifty-three schoolgirls who sometimes wore light leather sandals, the subjects were all habitually barefooted.

Details of the age distribution of the subjects are given below, in Tables 1 and 2. It falls into two main groups for each sex, one comprising children, most of whom were considered to be between 12 and 18 years of age, and a second comprising adults, most of whom were considered to be more than 35 years of age. It is difficult to obtain accurate information about age in African communities, so that the age distribution, particularly for the older adults, is subject to error, the magnitude of which it is hard to assess; it would not be unreasonable to regard the ages as accurate within  $\pm 5$  years, except perhaps in the age range 60–80 years, where error may be greater.

The European samples of both sexes are restricted mainly to young adults, most of whom were University students, nurses and teaching or research staff.



Fig. 1. Method of measuring foot length, breadth and Hallux angle on a footprint.

# RESULTS

Table 1. *Male subjects*

Sample	No.	Mean hallux angle	S.D.	Mean age (years)	S.D.
1. Europeans	66	+6.9°	5.3°	25.9	7.8
2. Nigerian soldiers	113	+2.2°	7.2°	—	—
3. Nigerians—25 years and older	108	+1.1°	6.8°	49.9	14.0
4. Nigerians below 25 years	106	+2.3°	5.8°	16.2	3.6
5. Nigerians—2 and 3 pooled.	221	+1.7°	7.0°	—	—
6. Total Nigerian males	327	+1.88°	6.6°	—	—

Table 2. *Female subjects*

Sample	No.	Mean hallux angle	S.D.	Mean age (years)	S.D.
1. Europeans	68	+11.0°	5.1°	21.4	3.3
2. Europeans (morbid group. Hallux valgus)	84	+21.9°	7.0	—	—
3. Nigerians—25 years and older	148	-0.03°	7.2°	45.5	9.8
4. Nigerians below 25 years	177	+0.49°	5.8°	15.5	2.7
5. Nigerians below 18 years—shoeless	120	+0.18°	6.1°	—	—
6. Nigerians below 18 years—shoes	53	+1.36°	5.1°	—	—
7. Total Nigerian females	325	+0.24°	6.5°	—	—

## COMPARISONS OF THE MEAN HALLUX ANGLE

The difference of 4.1° between the hallux angle of the European males and females is very highly significant ( $t=4.63$  for D.F. 132). The hallux in the females is deviated laterally to a greater extent than in males. Comparing first the African male series with the European males, the difference of angle of 5.8° between the Africans of 25 years and over and the Europeans is again highly significant ( $t=5.9$  for D.F. 172). The Europeans also differ very significantly from the Africans of less than 25 years whose age range is more closely comparable ( $t=5.20$  for D.F. 170).

In the young adult European male, therefore, the great toe is on average deviated laterally about 5.0° more than in African adults or adolescents.

Among the Africans themselves, comparison of the two rather widely contrasted age groups provides no evidence that the mean Hallux angle changes between adolescence and later adult life; the mean angle for a group of forty-eight subjects said to be more than 50 years of age was +1.73°.

The angle in the group of Nigerian soldiers who had worn army boots for a number of years was +2.21°, which is not significantly different from the angle of shoeless Africans of either age group. This sample was, therefore, pooled with that of shoeless Africans of 25 years and over for the purpose of further comparisons.

The mean hallux angle in African females differs even more from the European value than it does in males on account of the much greater lateral deviation of the toe in European females. The mean angle, which is close to 0°, does not differ



significantly between the two age groups of African females, nor it is significantly higher in the group of girls who had worn light footwear.

Pooling the various samples of African females, we obtain a total sample of 325 subjects, giving a mean angle of  $+0.24^\circ$  compared with a mean of  $+1.88^\circ$  for the pooled sample of African males. This small difference which is opposite in sign to that between the sexes in Europeans is statistically significant ( $t=3.2$  for D.F. 650).

The distribution of the hallux angle in a group of eighty-four European females who had presented themselves for operation on account of valgus deformity, is shown in Fig. 2; the mean angle was  $+21.87^\circ$ , which is clearly significantly different from the mean of either the European or Nigerian female samples. It will be noted, however, that there is considerable overlap of the distribution not only with the normal European one but also with the Nigerian.

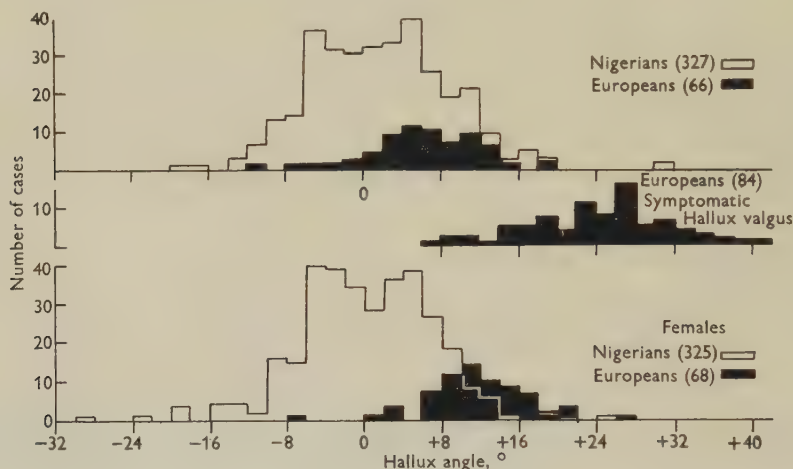


Fig. 2. Distribution of the Hallux angle in Europeans and Africans and in European females with symptomatic Hallux valgus.

#### THE VARIABILITY OF THE AFRICAN SAMPLES

Although, as we have shown, the mean values of the hallux angle do not differ significantly between the two age-groups in the Africans, there is a suggestion that the dispersion is greater in the older subjects than in the younger ones. The variance for the older group of males including the Nigerian soldiers ( $2+3$ ) is 48.99 and for the younger 33.23. Tested as the variance-ratio, the difference gives a value of  $F=1.47$  which, for D.F.  $n_1=221$  and  $n_2=106$ , is significant at 1.0%. Similarly, in the female samples the variance for the 25 years of age and over sample is 52.34 and for the younger group, 34.10, which again gives a significant  $F$  value. It will be noted that the male and female variances are substantially the same for each age group.

#### THE DISTRIBUTIONS OF THE HALLUX ANGLE

The distributions of the hallux angle for Africans and Europeans of both sexes are shown as histograms in Fig. 2. Since the Nigerian samples are quite large when pooled for each sex, the normality of the distribution can be examined. We have computed

the statistics for the 325 Nigerian females only. We find  $\gamma_1 = 0.375$  with approximate s.e. 0.134, indicating significant negative skewing. There is thus an excessive number of cases with extreme medial deviation of the hallux. Judging from the appearance of the distributions this tendency is less marked in the males. The distribution is also significantly leptokurtic, that is to say, both the positive and negative tails of the distribution contain more observations than is expected in a normal curve ( $\gamma_2 = 2.053$ , approximate s.e. 0.272). In both sexes the central region of the distribution appears to be remarkably flat. Since there is some evidence of a change in the degree of dispersion of the angle with age, these abnormalities of the pooled samples are probably in part related to this. It would be premature to attempt to interpret the shape of the distribution without a much larger material of varying age groups, but there is a suggestion that in the older ranges, the distribution is tending to become bimodal.

#### THE SIZE AND PROPORTIONS OF THE FOOT

It was felt worth while to see whether there was any notable difference between European and Nigerian samples in this respect. The mean values for the length and breadth are given below in Table 3. The comparison was made for subjects of 25 years of age and above only.

Table 3. *Dimensions of the footprint*

Sample	No.	Mean length (cm.)	Mean breadth (cm.)	Ratio breadth/length
Nigerian females	148	22.0	8.03	0.36
European females	68	22.6	8.32	0.37
Nigerian males	221	24.3	8.86	0.37
European males	60	25.0	8.94	0.36

The European measurements are slightly larger than the Nigerian ones for both dimensions and in both sexes. The proportions of the foot are, however, similar. We may note that the sample of 113 Nigerian soldiers gave a mean length of 24.9 cm. and a mean breadth of 8.94 cm., which are very close to the European male values and may be due to some selection for stature in recruiting.

There appears to be no clear relationship between the proportions of the foot and the hallux angle as seen by plotting the variables on a scatter diagram.

#### MISCELLANEOUS OBSERVATIONS

A wide gap between the first and second toe is commonly observed in Africans, and probably in most barefooted peoples. We measured the minimum distance between the imprints of these two toes in footprints from seventy-six of our Nigerian girls and found a mean value of 6.9 mm. compared with 4.9 mm. for sixty-six European females. Though we have not investigated the point fully, there does not appear to be a very close correlation between the hallux angle and the width of this interdigital gap. This is probably due in part to deviations of the 2nd and more lateral toes occurring in the same direction as the great toe, and perhaps also to variations in the degree to which the 2nd phalanx of the great toe is angulated on the 1st phalanx. In the course of collecting the material it was remarked that injuries to the great toe

and injuries and loss of lateral toes were frequent among the village people, but no systematic records were kept. Engle & Morton (1931) also noticed that loss of toes due to injuries and to yaws was very common among natives of the Belgian Congo. We may also note that although in some of our specimens the medial aspect of the sole of the foot touches the ground in a manner which Wells (1930-1) regards as characteristic for the South African Bantu, in the majority of cases the footprint is markedly indented on this aspect.

#### DISCUSSION

The outstanding findings are first, the large difference between the mean hallux angle in Europeans and Nigerians; and secondly, the virtual absence of the marked sex difference in this angle in Africans.

The mean hallux angle in the Nigerians is close to zero, and in this respect, may be compared with the small angle found in European infants of 4-6 years of age by Hardy & Clapham (1952) using radiographic methods.

The markedly greater valgus deviation in European females than males may reasonably be correlated with more constricting footwear and the very much smaller difference between the sexes in Africans tends to support this interpretation. We do not know whether the condition of the great toe shown in our African material would also occur in other barefooted peoples irrespective of ethnic affiliations. Wells (1930-1) states that in the South African Bantu and Bushman foot the first metatarsal is abducted but the first phalanx deviated laterally to a more valgus position than is usual in Europeans; he gives no measurements, however. Neither Engle & Morton (1931) nor James (1939), who examined the feet of Solomon Islanders, deal with this particular problem. Although yaws and probably gonorrhoea are common in Southern Nigeria, we have no clinical data on our subjects which would enable us to assess the contribution of these diseases to skeletal changes in the feet. Some footprints kindly sent us by Dr B. Kalcev of natives of Madagascar showed, however, a similar distribution of the hallux angle.

Although the mean hallux angle does not show much evidence of change with age in Africans, we have presented some evidence that deviations of the great toe both medially and laterally, become more extreme with advancing years. It would be reasonable to suppose that some variation of the hallux angle around zero may be present even at birth and that such pre-existing deviations tend to become exaggerated with increasing age. We have no definite proof of this interpretation, however, nor do we know the factors which produce the increased frequency of large deviations.

It will be noticed that although the mean angle of the hallux is considerably greater in a group of Europeans suffering from symptomatic hallux valgus than it is in normal Europeans, nevertheless, the distribution overlaps the range of angle of normal subjects of both sexes. A large lateral deviation of the toe is therefore not necessarily associated with clinical symptoms in all cases. To some extent the larger angle often associated with symptomatic bunion may be a result of the contact point *C* which is used in the measurement being displaced medially by the thickening in the region of the metatarso-phalangeal joint, thus giving a somewhat spurious impression of the angular deviation of the toe on the metatarsal. It appears that



only a long-term study on individuals could determine how far a large lateral deviation present from an early age increased the liability to symptoms. It seems unlikely from histories given by patients that particularly tight footwear can be the only factor which leads to pathological degrees of the condition.

The distribution of the angle in symptomatic Europeans not only overlaps the European normals but also the Africans, so that a considerable number of Africans have lateral deviation of the great toe equal to or even greater than those observed in many Hallux valgus cases. We have no evidence as to whether these larger deviations in Africans are associated with any discomfort or disability. Occasional valgus deviation of more than  $+20^\circ$  are sometimes found in barefooted Africans, and in both sexes some 20–30% of the sample falls within the European symptomatic range. Deviations of more than  $+10^\circ$  are not infrequent in Africans below 25 years of age, although they tend to increase in frequency in older age groups. It is clear that, whatever may be the cause of such deviations in Africans, footwear can play no part in their production.

#### SUMMARY

1. Evidence from footprints collected from 327 male Nigerians and 325 females is examined in relation to the position of the great toe and compared with European footprint material obtained from clinically normal subjects and those suffering from Hallux valgus.

2. The distribution of the hallux angle in the Nigerians of both sexes is around a mean of approximately zero which differs by a statistically significant amount from the mean values for European males and females which are  $+6.9^\circ$  and  $+11.0^\circ$  respectively.

3. The mean hallux angle is not significantly different as between the two age groups of Nigerians examined. There is evidence, however, of an increase in the dispersion of the values with age. A degree of valgus deviation falling well within the European pathological range occurs in some African subjects.

4. The results are discussed in relation to the causation of valgus deformity.

We are indebted to the District Officer, Ikeja Division, for assistance in obtaining samples from villages, and to Dr W. S. S. Ladell, Hot Climate Physiological Research Unit, Lagos, for allowing Mr E. A. Osinyemi to supplement some of our material. We also wish to acknowledge the loan of a Scholl Pedograph by the British Boot, Shoe and Allied Trades Research Association.

#### REFERENCES

- CRAIGMILE, D. A. (1953). Incidence, origin and prevention of certain foot defects. *Brit. med. J.* **2**, 749–752.
- ENGLE, E. T. & MORTON, D. J. (1931). Notes on foot disorders among natives of the Belgian Congo. *J. Bone Jt. Surg.* **13**, 311–318.
- HARDY, R. H. & CLAPHAM, J. C. R. (1951). Observations on Hallux valgus. *J. Bone Jt. Surg.* **33B**, 376–391.
- HARDY, R. H. & CLAPHAM, J. C. R. (1952). Hallux valgus. Predisposing anatomical causes. *Lancet*, **1**, 1180–1183.
- JAMES, C. S. (1939). Footprints and feet of natives of the Solomon Islands. *Lancet*, **2**, 1390–1393.
- WELLS, L. H. (1930–1). The foot of the South African native. *Amer. J. phys. Anthropol.* **15**, 185–289.

# THE FORM AND FUNCTION OF THE CARPO-METACARPAL JOINT OF THE THUMB

By J. R. NAPIER

*Department of Anatomy, Royal Free Hospital School of Medicine, London*

Although this joint has been extensively studied by many workers including Fick, L. (1854), Fick, R. (1911), Du Bois Reymond (1895, 1896), and more recently by Haines (1944), certain aspects of its motion in relation to the function of the thumb, and in particular to the part played by the joint in the complex movement of opposition, would seem to merit further investigation in the light of MacConaill's (1946*a-d*) studies of the fundamental mechanics of synovial joints.

The exact status of the term opposition is in itself a problem that cannot be solved by turning to the standard books of reference. Opposition is used here in a functional sense and is defined as that movement which results in the pulp surface of the thumb becoming diametrically opposed to the pulp surface of one or other of the remaining digits for the purposes of prehension.

The observations made are based upon examination of the joint in forty-two cadavers during the course of a previous investigation (Napier, 1952) and upon six dissections recently carried out.

## OBSERVATIONS AND DISCUSSION

### *The form of the articular surfaces*

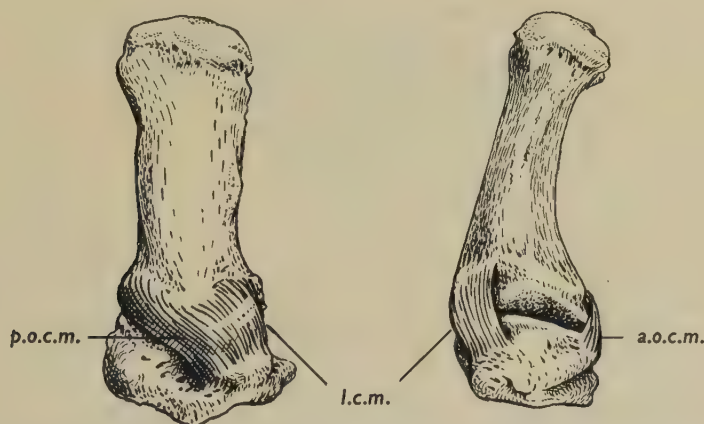
The carpo-metacarpal joint of the thumb is composed of two articulating surfaces which are concave in one principal section and convex in the other. The joint differs in one important respect from other saddle-shaped joints. Sections made of the ankle joint, for example, in the planes of its two principal curvatures demonstrate a relative congruity between the articulating surfaces when the joint is in the neutral position. Corresponding sections through the first carpo-metacarpal joint held in the neutral position reveal quite a different state of affairs. In the radio-ulnar section virtual congruity is apparent, but in the dorsi-palmar section a wide disparity is seen between the two bones (Text-fig. 4). The curvature of the metacarpal base is considerably greater than that of the distal surface of the trapezium, so that there exists between them a wedge-shaped interval that, in life, is filled partly by synovial fluid and partly by fibro-fatty synovial folds (Haines, 1944). It is upon this incongruity, together with the relaxed condition of the ligaments, that the well-known passive mobility of the joint in its mid-position depends (Fick, R., 1911). Attempts to rotate the thumb passively in a fully abducted or adducted position, on the other hand, meet with the resistance of congruous surfaces and tense ligaments.

### *Arrangement of ligaments*

Haines (1944) has given a comprehensive account of the ligaments of the first carpo-metacarpal joint and his observations have been, in the main, confirmed in the present series of dissections. Special mention, however, should be made of the

lateral carpo-metacarpal ligament which consists of vertical fibres attached to the postero-lateral aspect of the metacarpal and to the posterior tubercle of the trapezium (Text-fig. 1); and of the posterior oblique carpo-metacarpal ligament whose fibres reach their distal attachment to a tubercle on the medial side of the metacarpal after an oblique course.

Haines (1944) suggests that these two ligaments are discrete, but in the six dissections of the present series their fibres were found to merge at their edges.



Text-fig. 1. Dissection showing the position and attachments of the three main ligaments of the carpo-metacarpal joint of the left side: (a) posterior view, (b) lateral view. *p.o.c.m.*, posterior oblique carpo-metacarpal ligament; *l.c.m.*, lateral carpo-metacarpal ligament; *a.o.c.m.*, anterior oblique carpo-metacarpal ligament.

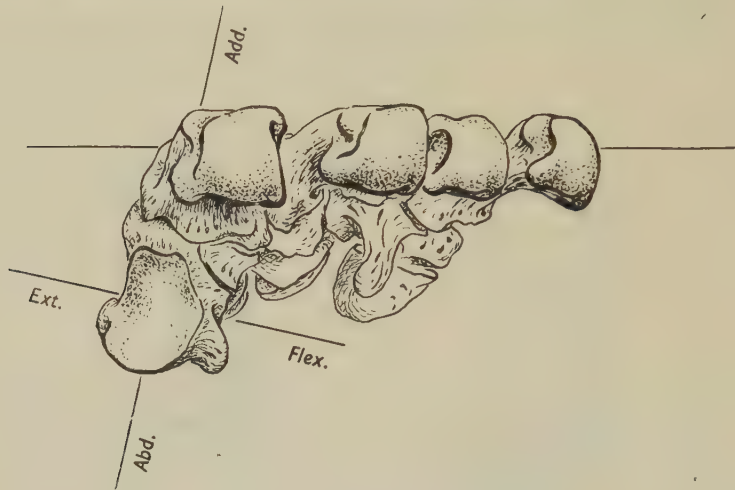
### *Motion in the joint*

The neutral position of the thumb metacarpal is taken as the starting-point for a description of the movements; this not only simplifies description, as Haines (1944) has observed, but is an entirely logical starting-point since it corresponds to the position of rest of the thumb in the living hand. In the account which follows, the movements of the metacarpal are defined in terms of the axes of the distal articular surface of the trapezium rather than directly in terms of the plane of the palm. The trapezium is set in the carpus in such a manner that the long axis of its distal articular surface makes an angle of  $80^{\circ}$  with the plane of the palm (Text-fig. 2).

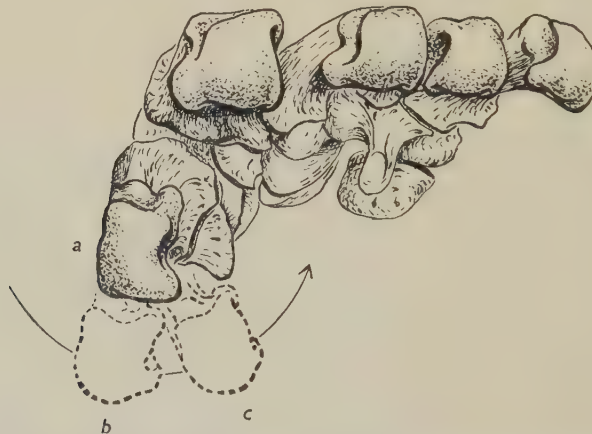
Thus, in the angular movement of abduction the metacarpal moves away from the index finger in a somewhat antero-lateral direction, making an angle of  $80^{\circ}$  to the plane of the palm. During flexion, which takes place in the plane of greatest convexity of the trapezium, the thumb passes medially and  $10^{\circ}$  forwards of the plane of the palm. The movements of flexion and extension are sliding movements of the metacarpal on the trapezium with a somewhat limited range. For present purposes it is sufficient to consider both these movements as uniform translations along a chord, and they are, therefore, by definition, movements free from any associated rotation. This can be shown to be true in an osteo-ligamentous preparation providing abduction is prevented.



The movements of abduction and adduction take place in a plane which corresponds to the greatest concavity of the trapezium which is at right angles to the plane of flexion-extension. Abduction-adduction movements are brought about mainly by a sliding of the metacarpal on the trapezium and they, too, can be regarded



Text-fig. 2. The carpal and metacarpal bones of the left hand seen in vertical view. The 1st metacarpal has been omitted to expose the distal articular surface of the trapezium. The planes of flexion-extension (*Flex.-Ext.*) and abduction-adduction (*Abd.-Add.*) are indicated.



Text-fig. 3. The view is the same as in Text-fig. 2, but in this case the 1st metacarpal is in place. *a*, metacarpal head in neutral position; *b*, in abduction; *c*, in full medial rotation.

as uniform translations, for they are unassociated with any rotation when flexion or extension are prevented. The axes round which these angular movements occur are illustrated by Toldt (1901) in fig. 389 of his Atlas.

As the metacarpal is abducted, the lower end of the bone is displaced in a dorsal direction towards the index metacarpal until a relative congruity of the surfaces is

established (Text-fig. 4). Similarly, the opposite displacement of the base of the first metacarpal, that is to say in a direction away from the index metacarpal, occurs during adduction (Text-fig. 4). During this movement in the living hand an obvious prominence appears on the radial border of the hand (Pl. 1, fig. 1) and disappears when the thumb is returned to the neutral position. This gross displacement of the metacarpal base reflects the extent of the sliding movement which converts what is virtually a point contact into the state of relative congruity that obtains in abduction. When the thumb is fully abducted, it may be freely circumducted\* in either an ulnar or a radial direction: movement in an ulnar direction is associated with medial rotation of the metacarpal, and movement in a radial direction with lateral rotation.



Text-fig. 4. Diagrams of a longitudinal section of the 1st left carpo-metacarpal joint made in the abduction-adduction plane. Note the incongruity apparent in the mid-position.

During each of these movements the metacarpal traverses, in effect, a quadrant of the total pathway of circumduction. The pathway traversed by the head of the metacarpal consequent upon an abduction followed by a circumduction displacement of the base can be regarded as a movement starting from the centre of a circle, passing along the radius to the circumference and then along the circumference itself. The second part of its course necessarily involves a change in direction of the head. This two-part movement is an example of a *diadochal* movement (Text-fig. 3, *a-b-c*). MacConaill (1946*b*) has shown mathematically that a diadochal movement at any joint is necessarily accompanied by a degree of axial rotation at the articular surface, that, in terms of the generalized geometry of curved surfaces, is known as a *conjunct* rotation. Thus, in the carpo-metacarpal joint of the thumb, medial rotation of the metacarpal is a conjunct rotation and is the *inevitable accompaniment of abduction followed by circumduction in an ulnar direction*.

The serial photographs in Pl. 1, figs. 2-5, illustrate, in a functional manner, this movement of circumduction in a living hand. In Pl. 1, fig. 2, the thumb is shown fully abducted at the carpometacarpal joint, but, although it is functionally opposing

\* Circumduction is here used to describe that form of motion that takes place between the head of a bone and its articular cavity when the bone is made to circumscribe a conical space (Gray's *Anatomy*, 1954 edition). It is not used in the theoretically general sense in which MacConaill (1946*b*) employs it.

the 5th digit, it has not yet undergone any measureable medial rotation at this joint; the rotation of the thumb apparent in this photograph is taking place at the metacarpophalangeal joint and not at the carpo-metacarpal joint. In Pl. 1, fig. 5, the thumb is shown in opposition to the index finger, its metacarpal now being both fully abducted and fully medially rotated. The thumb can attain this terminal posture by traversing the circumduction pathway through the series of stages illustrated, but it can also achieve it by following an alternative and more direct pathway which might be described as a short cut. Starting from a position of rest (the mid-position of the joint) the thumb may move *by a direct route* to a posture where it is in opposition to the index finger (Text-fig. 3, *a-c*) without having traversed the intermediate stages. This latter movement may be visualized by sliding the thumb distally from its position of rest opposite the terminal interphalangeal joint of the index finger until the tip of the finger is reached. This movement, which is in fact the habitual one in the normal hand when objects of small size are handled, consists of a movement of medial rotation combined with flexion and abduction.

The behaviour of the ligaments of the carpo-metacarpal joint of the thumb in an osteo-ligamentous preparation provides evidence in support of the reality of these two alternative pathways. In the neutral position all ligaments of the joint are relaxed. As the thumb metacarpal is moved through the first leg of the diadochal pathway (Text-fig. 3, *a-b*), that is to say when it is abducted, the posterior oblique ligament becomes tense, and the lateral ligament becomes further relaxed. During the second leg of the movement (Text-fig. 3, *b, c*)—the circumduction component—axial rotation of the bone occurs inevitably with the application of a *flexing* force. The posterior oblique ligament remains tense throughout this phase. During the short-cut movement, on the other hand, medial rotation is not inevitable and requires the application of a *rotatory* force to bring it about. During this movement the posterior oblique ligament remains lax until the rotation is nearly complete, at which time tension develops. This tension does not, however, develop as a direct result of medial rotation or again of flexion, as Haines (1944) has suggested, but as a result of the abduction component of the short-cut movement which effects a separation of the bones on the side nearest the index finger and, therefore, a separation of the extremities of the ligament. In fact, throughout simple flexion the posterior oblique ligament remains relaxed.

Thus, in an osteo-ligamentous preparation, full medial rotation can be brought about by movement of the metacarpal through two different pathways to reach the same end-point: (1) an *indirect* movement consisting of abduction followed by circumduction and accompanied by medial rotation; (2) a *direct* movement of medial rotation, accompanied by flexion and abduction. During the indirect movement the ligaments of the joint manifestly contribute to the rotation while in the direct movement they remain relaxed until the movement is virtually complete.

Thus it would appear that the rotation of the metacarpal, which occurs during the direct movement, must depend largely upon the existence of a special rotator muscle. The opponens pollicis would seem to be admirably situated in this respect. Such a movement is termed an *adjunct* rotation (MacConaill, 1946*b*), and is made possible in this instance by the incongruity of the articular components of the joint, discussed above. At the extreme of the direct movement, however, when congruity



is finally established, it is probable that an element of conjunct rotation is present, but its effect is insignificant in the presence of the well-marked adjunct rotation.

Fick, R. (1911) points out that in spite of the saddle-form of the articular surfaces longitudinal rotations are not impossible, owing to the loose composition of the joint. He does not make it clear, however, that such rotations are only possible when the joint is in its mid-position. Theoretically, the direct or short cut movement, which is essentially a longitudinal rotation, can only occur in the mid-position when the laxity of the joint and the incongruity of the articular surfaces favour such a movement.

The following case history of a patient who has been under personal observation for 10 years provides some evidence in support of these anatomical observations by demonstrating that paralysis of the opponens pollicis does not abolish the mechanism of medial rotation of the metacarpal:

G. F. C., age 39, male. Gunshot wound. R. arm, resulting in a median nerve paralysis. Nerve exploration revealed 8 cm. gap in median nerve. Suture was not attempted. When examined 7 years after injury the abductor pollicis brevis and opponens pollicis were completely paralysed but the flexor pollicis brevis was acting strongly (Pl. 1, fig. 6). The functional opposition of the thumb was not perfect (Pl. 1, fig. 7) owing to the loss of abduction and rotation of the proximal phalanx, but the metacarpal could be fully abducted and medially rotated by the combined action of the abductor pollicis longus and flexor pollicis brevis.

The patient is only able to achieve opposition by the indirect movement. Abduction is achieved by the contraction of abductor pollicis longus, while the flexor pollicis brevis provides the angular force necessary to circumduct the metacarpal in an ulnar direction. The medial rotation accompanying the circumduction movement is clearly quite independent of the opponens pollicis muscle which, in this case, was paralysed.

#### *Functional considerations*

Duchenne (1856) records that on electrical stimulation of the abductor and flexor pollicis brevis, the movement which preceded opposition depended on the posture of the thumb at the time of stimulation. When the thumb was stimulated in its 'natural position' (*sic*) the movement followed the shortest course, but when the thumb was initially abducted, opposition was preceded by a sweeping movement of the thumb which he describes as 'a sort of circumduction'. It is clear from this description that although Duchenne recognized the existence of two alternative pathways he was not, apparently, aware of their functional significance.

The characteristic requirement of prehensile movements of the hand is that of stability. In respect of the thumb this stability is achieved by full abduction or full adduction at the carpo-metacarpal joint; either action renders the articular surfaces congruent. In movements in which the demands of precision are paramount and the need for power is of secondary importance, the thumb is fully abducted at the carpo-metacarpal joint. If, on the other hand, the need for power is paramount the thumb is fully adducted at this joint (Napier, 1953). During precision movements, the thumb provides the requisite stability to the grip by opposing one or other of the digits. This state of opposition can be achieved, in respect of the metacarpal, by

movement along either of two pathways. In following the *indirect* pathway the thumb undergoes, first, abduction at the carpo-metacarpal joint, followed by circumduction in an ulnar direction, a movement which has the effect of increasing the degree of axial rotation of the first metacarpal and, at the same time, reducing the span of the hand. Clearly there is a need, therefore, for a precise correlation between circumduction and axial rotation throughout the whole range of movement, so that the hand may accommodate itself to objects of different sizes without losing opposition and, thereby, sacrificing stability. Such perfect integration does not depend upon the function of opposing groups of rotatory muscles, as would be the case were the joint of the ball-and-socket variety, but upon the reciprocal congruence of the articular surfaces and a suitable arrangement of ligaments. Once the first metacarpal is abducted it is guided into the requisite degree of medial rotation as ineluctably as a child is conducted down the spiral way of a helter-skelter.

Alternatively, opposition of the thumb may be achieved by moving the first metacarpal through the *direct* pathway. The axial rotation in this case is not attributable to the possession of congruent articular surfaces and a tense posterior oblique ligament, but to incongruent surfaces and a complete laxity of all ligaments. The movement, it is suggested, is effected by a special rotator muscle—the *opponens pollicis*. This direct movement is habitually employed when objects small enough to be stabilized between finger and thumb are handled. The wide excursion of the metacarpal through the indirect pathway (Pl. 1, figs. 2–5) is necessarily employed when picking up an object too large to be held conveniently between finger and thumb.

#### SUMMARY

1. The observations are based on the examination and dissection of the first carpo-metacarpal joint in forty-eight dissecting room and post-mortem subjects.

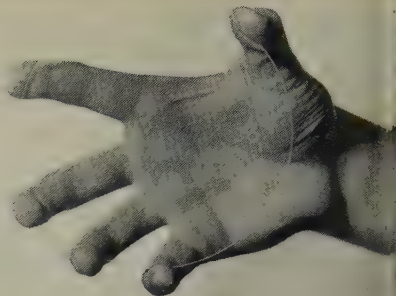
2. An account is given of the contours of the joint's surfaces and the arrangement of ligaments. In the mid-position of the joint the articular surfaces are markedly incongruent and the ligaments are relaxed, conditions which favour the well-known passive mobility of the joint in this position. Attempts to rotate the thumb passively in a fully abducted or adducted position, on the other hand, meet with the resistance of congruous surfaces and tense ligaments.

3. Motion in the joint is described, particularly the movements of the metacarpal which result in medial (axial) rotation of the bone. The significance of these movements is discussed in the light of MacConaill's (1946*a-d*) studies on the fundamental mechanics of synovial joints. It is suggested that there are two alternative pathways—here termed *direct* and *indirect*—by which the metacarpal may move into its fully rotated position, the former being dependent upon the loose composition of the joint in its mid-position, and the latter upon the state of congruity that exists between the articular surfaces when the joint is abducted.

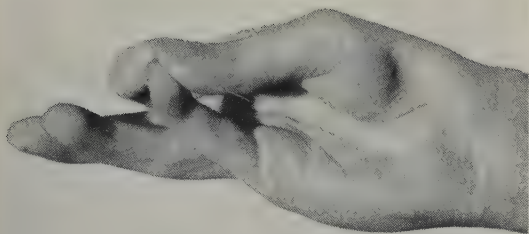
4. The functional significance of these alternative pathways are discussed in terms of the function of the hand as a whole. It is suggested that the selection of the appropriate movement in the normal living hand is dependent mainly upon the dimensions of the object to be handled.



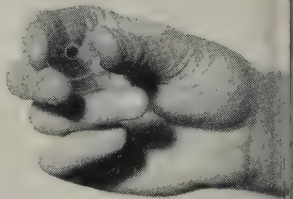




6



7



NAPIER—FORM AND FUNCTION OF THE CARPO-METACARPAL JOINT OF THE THUMB

I am indebted to Prof. M. A. MacConaill and Mr C. H. Barnett, F.R.C.S., and Prof. Ruth E. M. Bowden, for their valuable advice. Mrs A. Besterman was responsible for the drawings and Mr J. F. Crane for the photographs.

#### REFERENCES

- DU BOIS-REYMOND, R. (1895). *Arch. Anat. Physiol.* (Physiol. Abt.).  
DU BOIS-REYMOND, R. (1896). *Arch. Anat. Physiol.* (Physiol. Abt.).  
DUCHENNE, G. B. (1856). *Physiology of Motion*. (English translation by Kaplan, 1949.) Philadelphia: Lippincott.  
FICK, L. (1854). Quoted by Fick, R. (1911).  
FICK, R. (1911). *Handbuch der Anatomie und Mechanik der Gelenke*, Part III, 401-405. Jena: Fischer.  
HAINES, R. W. (1944). Mechanism of rotation at the first carpo-metacarpal joint. *J. Anat., Lond.*, 78, 44-46.  
MACCONAILL, M. A. (1946*a*). Some anatomical factors affecting the stabilising functions of muscles. *Irish J. Med. Sci.* May, pp. 1-5.  
MACCONAILL, M. A. (1946*b*). Studies in the mechanics of synovial joints. I. Fundamental principles and diadochal movements. *Irish J. Med. Sci.* June, pp. 190-199.  
MACCONAILL, M. A. (1946*c*). Studies in the mechanics of synovial joints. II. Displacements on articular surfaces and the significance of saddle joints. *Irish J. Med. Sci.* July, pp. 223-235.  
MACCONAILL, M. A. (1946*d*). Studies in the mechanics of synovial joints. III. Hinge-joints and the nature of intra-articular displacements. *Irish J. Med. Sci.* September, pp. 620-626.  
NAPIER, J. R. (1952). The attachments and function of the abductor pollicis brevis. *J. Anat., Lond.*, 86, 335-341.  
NAPIER, J. R. (1953). The prehensile movements of the human hand. *Anat. Rec.* 115, 2, 352.  
TOLDT, C. (1901). *Anatomischer Atlas für Studierende und Ärzte*, 2nd ed., fig. 389, p. 167. Berlin and Vienna: Urban and Schwarzenberg.

#### EXPLANATION OF PLATE

- Fig. 1 Radiograph of normal hand taken in the A.P. plane of first metacarpal which is fully adducted. Note the extent of the lateral displacement of the base of the bone.  
Figs. 2-5. The photographs illustrate in a living hand the movement of circumduction of the thumb in an ulnar direction taking place at the carpo-metacarpal joint. The thumb is in opposition with one or other of the digits throughout the series.  
Fig. 6. Photograph of the right hand of a patient (G. F. C.) with a high division of the median nerve. Abductor pollicis brevis and opponens pollicis are paralysed but flexor pollicis brevis is active.  
Fig. 7. The same patient performing thumb to little finger opposition. Abduction and rotation of the proximal phalanx of the thumb is not complete, however, owing to the paralysis of the abductor pollicis brevis.

# THE THORACO-LUMBAR MORTICE JOINT

By P. R. DAVIS

*Royal Free Hospital School of Medicine, London*

## INTRODUCTION

There are many recorded observations on the anatomy of the vertebral column. Much of this work has been done with comparative, and some with human, material. Amongst the detailed studies of the different regions in man are those of Humphry (1858), Holden (1861), Macalister (1889), Frazer (1940), and Slijper (1946), and a clear picture has been drawn of the anatomy and functions of the thoracic and lumbar regions as separate units.

The thoraco-lumbar junctional region has, however, received less attention. Humphry stated that the lower two dorsal and upper one or two lumbar vertebrae were the weakest part of the column, a view endorsed by Holden. Macalister stated that the point most exposed to injury was the union of the dorsal and lumbar curves. These three authors suggested that this vulnerability was due to the junction of the relatively immobile thoracic column with the mobile lumbar region. In addition, Humphry suggested that the bodies of these vertebrae were relatively too small to bear the weight upon them, that the transverse and spinous processes were too short to give adequate muscular support, and that the position of the region within the middle of the column exposed it to maximum leverage from above and below. No supporting evidence was given for these views, however, nor were these various factors collated in individual columns. A report of a further study of the osteology of this portion of the vertebral column is, therefore, justifiable.

## MATERIAL

Out of a series of 149 dry adult human vertebral columns, sixty-nine were chosen for this study since they were free from artefacts and pathological changes. Twenty-two younger specimens, both dry and cartilage covered, were also studied. Their ages ranged from full term to 20 years. Not all these younger specimens were complete columns, but all used for this investigation had at least the lower eight thoracic and upper four lumbar vertebrae. Five fresh adult specimens of the lower thoracic and upper lumbar region were used to study movements, one from a female of 21 years, the others from males of 33, 34, 34 and 36 years respectively.

## METHODS

The configuration of the articular processes was carefully noted in all specimens. In fourteen columns the areas of the upper surfaces of the vertebral bodies were measured. Their outline was traced on to standard six-sheet card and this outline was cut out and weighed, its area being calculated from the known weight-to-area relationship of the card. In the same fourteen columns pedicle thickness was assessed by measuring with callipers the greatest and least diameters of the most slender



portion of the pedicle. These two figures, in millimetres, were then multiplied together, and the product termed the *pedicle index*. This index is, therefore, a rough guide to the cross-sectional area of the pedicle at its most slender part. In all columns, particular attention was paid to the markings on the laminae.

## OBSERVATIONS

### *Articular processes*

In sixty-seven of the sixty-nine adult columns, one zygapophyseal joint at the thoraco-lumbar transitional region differed markedly from those above and below it. Processes of the lower participating vertebra combined to form a structure comparable with a carpenter's mortice (Pl. 1). The mortice was bounded anteriorly by the two superior articular processes, laterally by the combined transverse and mammillary processes (superior tubercles), and posteriorly by the overhanging mammillary processes alone. The lateral walls were closer to each other below than above. The tenon consisted of the inferior articular processes of the upper vertebra together with their connecting laminae, and was also narrower below than above.

In the twenty-two juvenile columns no evidence of the mortice was seen in the four below the age of 2 years, but it was found in sixteen of the remaining eighteen, including one aged 2 years and 8 months.

The level of the mortice joint was found to vary, but in the adult series was commonest between the eleventh and twelfth thoracic vertebrae:

Level of mortice joint	No. of columns
T 10-11	5
T 11-12	46
T 12-L 1	16
Total	67

(No figures are given for the level of the joint in the juvenile series as some of the columns were incomplete.)

In every case of both series the joint above the mortice was of the thoracic type, and that below it, lumbar. In the remaining six columns without a mortice, the twelfth thoracic vertebra had superior articular processes of a thoracic type, the inferior processes being of lumbar morphology.

The mortices varied in depth, and could be divided into three groups (Pl. 1). In the first of these, the lateral wall of the mortice fully enclosed the tenon, in the second it was less extensive but enclosed more than half, and in the third, less than half. The results were as follows:

	Adult	Juvenile
Group I	8	1
Group II	32	8
Group III	27	9
No mortice	2	4
Total	69	22

In groups I and II the articular surface spread on to the lateral walls for a varying distance; in group III it was restricted to the anterior wall. The extent of the articular surfaces of the tenons varied accordingly.

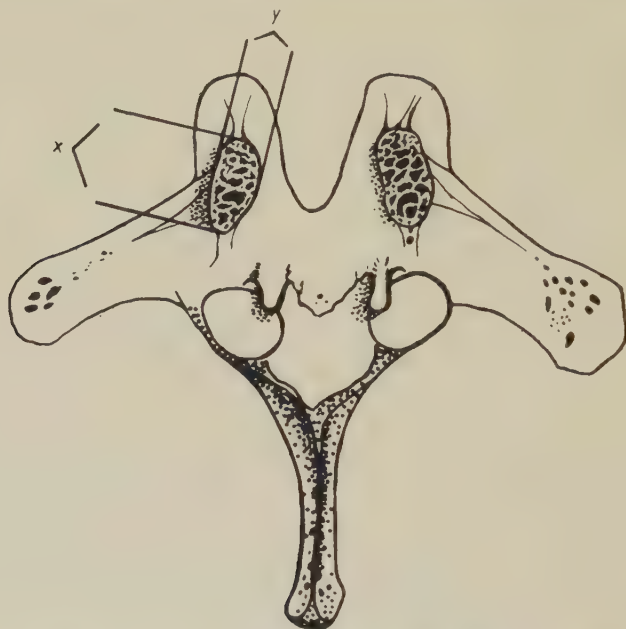
In the wet specimens, a very small movement of the tenon vertebra was sufficient

to lock the tenon into the mortice, thus preventing all movement other than flexion. If, with the mortice locked, a strong vertical compression force was applied to the vertebrae, the only movement was one of flexion; whereas at the thoracic or lumbar joints, the same force caused the vertebral bodies to approach each other slightly as the disc compressed, but without any other movement.

### *Planimetry*

The areas of the upper surfaces of the bodies of the thoracic and lumbar vertebrae were measured in ten adult and four juvenile columns.

A similar configuration was found in every column, that is, the areas increased slowly in the upper thoracic region, and then rapidly to the mortice vertebra, and



Text-fig. 1. A diagram of the ventral surface of the neural arch of a thoracic vertebra.

The pedicles were cut transversely at their waist. Pedicle index =  $xy$  (in mm.).

more slowly thereafter. The greatest rate of increase occurred in the mortice vertebra and those immediately above it, irrespective of the position of the mortice within the column.

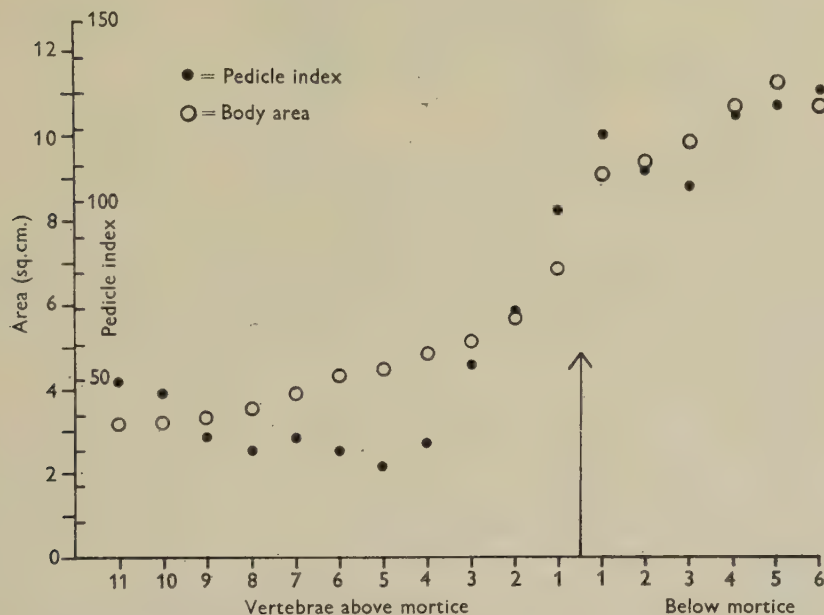
### *Pedicle index*

In the same fourteen columns, the pedicle indices were calculated for one pedicle of each thoracic and lumbar vertebra. In eight columns the pedicles of the right side were measured, in six, those of the left side (Text-fig. 1). In all columns the findings were similar. There was a fall in the upper thoracic region, a rise below this, with a sudden large increase as the mortice was approached and reached (Text-fig. 2). Below the mortice the index usually decreased slightly.

*The laminae*

In all columns the ventral surfaces of certain laminae bore a spicule or spur of bone within the marking for the upper attachment of the ligamentum flavum. These spicules sprang from the lateral part of the marking, and pointed inferiorly (Text-fig. 3).

In most columns they were present in several successive vertebrae, increasing in size from above downwards. The largest seen in this series were 5 mm. long, and 4 mm. wide at their base. The number and distribution of these spicules is shown



Text-fig. 2. The areas of the upper surfaces of the vertebral bodies, and the left pedicle indices, of a male adult vertebral column. The arrow marks the position of the mortice joint.

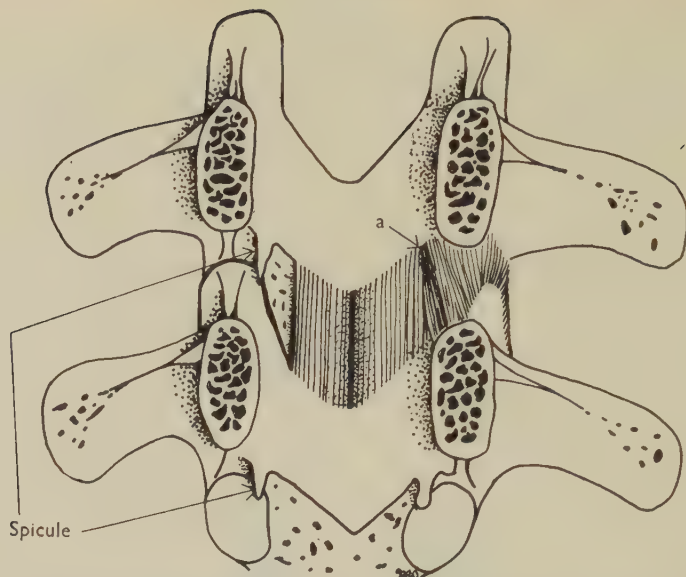
(Text-fig. 4) as a histogram, the vertebrae being numbered from the mortice joint, whence it can be seen that the spicules occurred with increasing frequency down to that level, and were but rarely present below it.

In this series the youngest column possessing these spicules was from a girl of 16 years, and spicules were also present in columns of 18, 20 and 21 years.

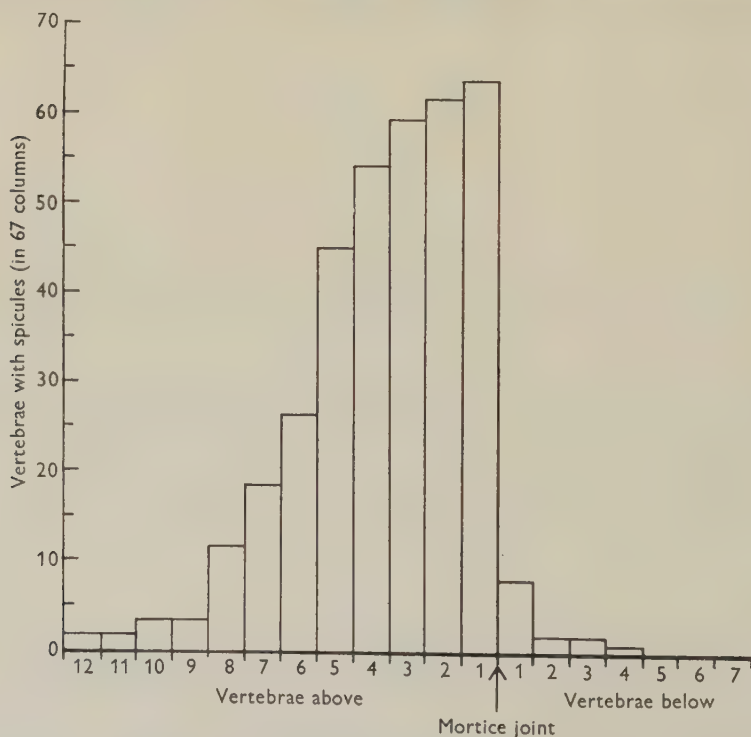
## DISCUSSION

Since the mortice joint intervenes between two series of zygapophyseal joints manifesting respectively thoracic and lumbar structural characters, it is reasonable to consider it as marking the junction of two functionally different regions of the spinal column. It is important to note that it may be placed above the lowest rib-bearing vertebra.





Text-fig. 3. A diagram of the ventral surfaces of the neural arches of two lower thoracic vertebrae. The ligamentum flavum and capsular fibres of the zygapophyseal joint are shown. *a*, the position of the spicule within the junction of the oblique capsular and the vertical flaval fibres.



Text-fig. 4. A histogram showing the frequency of laminar spiculation in sixty-seven adult vertebral columns.

It has been shown that, with the mortice locked, a compressing force, which is prevented by the lock from forcing the tenon downwards, must cause flexion of the joint, by compressing the intervertebral disc. This compression must raise the pressure within the nucleus pulposus, and hence increase the pressure on the vertebral bodies of the mortice vertebra and those below it.

The progressive increase in area of the vertebral bodies from above downwards must reflect the increasing load borne by each successive vertebra, realizing that this load is made up of the body weight, and any additional weight borne by the upper part of the body. In addition, the leverage that this load can exert on the individual thoracic and lumbar vertebrae increases steadily down to the pelvis. (In three of the fourteen columns measured, the last lumbar vertebra had an area somewhat less than that of its fellows (Text-fig. 2), together with large transverse processes. It seems probable that, in these, rather more weight than usual was carried by the ilio-lumbar ligaments.)

It is suggested that the marked increase of body area, related constantly to the level of the mortice, is a necessary reinforcement to sustain the great increase in pressure caused by the locked and flexed mortice joint. This compressing force will also tend to shear the neural arch from the body of the vertebra. The consequent need for reinforcement of the pedicles is reflected by their great increase in thickness.

The spicules found upon the ventral surfaces of the laminae have been described by Shore (1931) in the Bantu, and by Allbrook (1954) in East Africans. In this series they were found in every normal adult, and in several young, columns. They were of smooth bony structure, and appeared to be integral parts of the vertebrae, in no way resembling exostoses. These findings strongly suggest, but do not prove, that they are normal anatomical structures. Le Double (1912) thought that they were vestiges of zygosphenes, but the observations of Naffziger, Inman & Saunders (1938) refute this, for in their studies on the ligamentum flavum they found that the spicules lay within the ligament, at the point where its fibres intermingled with the obliquely placed fibres of the capsule of the zygapophyseal joint (Text-fig. 3). These oblique fibres are tightened by, and limit, thoracic axial rotation. The distribution of the spicules suggests that this limitation is greatest just above the mortice joint, the mortice itself allowing no rotation at all when locked.

In industrial accidents, crush fractures of the vertebral bodies most frequently involve the twelfth thoracic or first lumbar vertebra (Jefferson, 1927; Nicoll, 1949; Newman, 1952). These injuries are frequently caused by the descent of a large weight upon the head and shoulders of the individual, imposing a vertical compression force upon the vertebral column from above. In a large series of American parachuting injuries, Ciccone & Richman (1948) found that the majority of crush fractures also involved the twelfth thoracic and first lumbar bodies, hard falls in the upright posture imposing a vertical compression force from below. At a British parachute training establishment, the present author found that, of seven cases of crush fracture recorded, those four who had sustained large vertical deceleration had crushed the bodies of either the twelfth thoracic or first lumbar vertebrae. Such a distribution of fractures can be explained by the great increase of pressure that must follow the forced flexion of the locked mortice joint when such compression forces are applied.

## SUMMARY

The region of functional transition between the thoracic and lumbar regions of the human vertebral column is commonly marked by a type of zygapophyseal joint comparable with a carpenter's mortice. The areas of the vertebral bodies and the size of the pedicles increase most rapidly in this region. Laminae spicules within the ligamenta flava have a distribution which is related to the level of the mortice. The functional significance of these findings is discussed.

The author's gratitude is due to Profs. J. D. Boyd, A. J. E. Cave, D. V. Davies, W. J. Hamilton, R. J. Harrison, R. J. Last, E. W. Walls and J. Whillis, for kindly allowing access to material, and to the personnel of the Army Air Transport Training and Development Centre, in particular to Squadron-Leader J. G. Millar, for allowing him to see parachute descents. Prof. R. E. M. Bowden is thanked for her patient criticism and advice. The photographs were taken by Mr J. Crane. Gratitude is also due to the Wellcome Foundation.

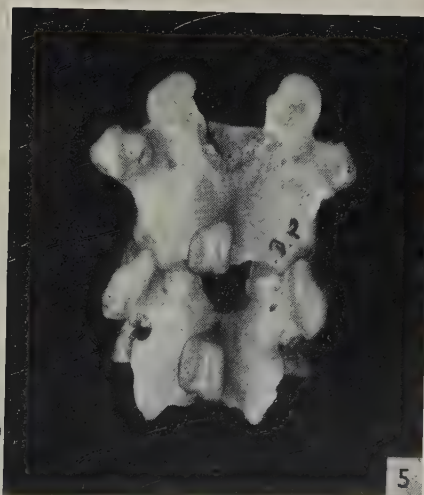
## REFERENCES

- ALLBROOK, D. B. (1954). Characteristics of the East African vertebral column. *J. Anat., Lond.*, **88**, 559.
- CICCONE, R. & RICHMAN, R. M. (1948). The mechanism of injury and the distribution of three thousand fractures and dislocations caused by parachute jumping. *J. Bone Jt. Surg.* **30A**, 77-97.
- FRAZER, J. E. (1940). *The Anatomy of the Human Skeleton*, 4th ed. London: J. and A. Churchill Ltd.
- HOLDEN, L. (1861). *Human Osteology*. 3rd ed. London: J. Churchill.
- HUMPHRY, G. M. (1858). *A Treatise on the Human Skeleton*. Cambridge: Macmillan and Co.
- JEFFERSON, G. (1927). Discussion on spinal injuries. *Proc. R. Soc. Med.* **21**, 625-637.
- LE DOUBLE, A.-F. (1912). *Traité des variations de la colonne vertébrale de l'homme*. Paris: Louis Danty-Collas.
- MACALISTER, A. (1889). *A Textbook of Human Anatomy*. London: C. Griffin and Co.
- NAFFZIGER, H. C., INMAN, V. & SAUNDERS, J. B. DE C. M. (1938). Lesions of intervertebral disc and ligamenta flava; clinical and anatomical studies. *Surg. Gynec. Obstet.* **66**, 288-299.
- NEWMAN, P. H. (1952). Sprung back. *J. Bone Jt. Surg.* **34B**, 30-37.
- NICOLL, E. A. (1949). Fractures of the dorso-lumbar spine. *J. Bone Jt. Surg.* **31B**, 376-394.
- SHORE, L. R. (1931). A report of the nature of certain bony spurs arising from the dorsal arches of the thoracic vertebrae. *J. Anat., Lond.*, **65**, 379-387.
- SLIJPER, E. J. (1946). Comparative biologic-anatomical investigations on the vertebral column and spinal musculature of mammals. *Verh. Akad. Wet. Amst. B*, **42**, 1-128.

## EXPLANATION OF PLATE

- Fig. 1. Superior view of a twelfth thoracic vertebra from a male adult, showing the components of a group I mortice. The mammillary processes overhang the joint cavities posteriorly; their roots, together with the roots of the transverse processes, form the lateral walls, and anteriorly the articular processes are seen. The lateral walls approach each other in the lower, deeper, parts of the mortice. The articular surface lines all three walls.
- Fig. 2. A dorsal view of the eleventh and twelfth thoracic vertebrae, from the same column as fig. 1. The tenon, formed by the inferior articular processes of the eleventh thoracic vertebra and their connecting laminae, is narrower below than superiorly. The mortice in the lower vertebra shows the overhang of the mammillary processes.







- Fig. 3. Dorsal and lateral views of the eleventh and twelfth thoracic vertebrae of a male adult, showing a group I mortice (the same vertebrae as in fig. 1). The inferior articular processes (tenon) are fully enclosed laterally by the mortice of the lower vertebra.
- Fig. 4. The tenth and eleventh thoracic vertebrae of a female adult, showing a group II mortice. Half of each of the inferior articular processes of the upper vertebra are enclosed laterally by the mortice.
- Fig. 5. The eleventh and twelfth thoracic vertebrae of a male adult, showing a group III mortice. Only the lowest parts of the inferior articular processes of the upper vertebra are enclosed laterally by the lateral wall of the mortice.



# THE ROLE OF THE SCALENE AND STERNOMASTOID MUSCLES IN BREATHING IN NORMAL SUBJECTS. AN ELECTROMYOGRAPHIC STUDY\*

By E. J. M. CAMPBELL

*The Department of Physiology,  
The Middlesex Hospital Medical School, London*

## INTRODUCTION

The activity of all the muscles which are generally thought to act as accessory muscles of inspiration has been examined electromyographically in normal men. The only ones which showed significant respiratory activity were the sternomastoid and the scaleni; these were investigated more fully. It is well known that these muscles are important accessory muscles of inspiration in dyspnoeic subjects, but their role in normal subjects has not apparently been examined hitherto.

## METHODS

*Apparatus.* An Ediswan amplifier with inkwriter oscillographs was used. The surface electrodes used were shallow cups of silver coated with silver chloride. A 6 l. water-filled Kendrick spirometer was arranged to give a record of the respiration on the electromyograph paper.

The apparatus described by Campbell & Green (1953) for studying graded expiratory efforts was modified to allow inspiratory efforts also to be studied. This apparatus consists of a differential manometer in which any desired pressure can be maintained by the observer, and which is balanced by a voluntary inspiratory (or expiratory) effort made by the subject.

*Subjects.* Five healthy young men aged 18–27 were studied. The muscles on the right side only were examined.

*Application of the surface electrodes.* The skin was rubbed first with ether and then with Cambridge electrode jelly to reduce skin resistance. The cups of the electrodes were filled with electrode jelly and then fixed to the skin with collodion or adhesive plaster.

### *Placing of the electrodes*

*The sternomastoid.* A pair of electrodes was placed 4.5 cm. apart along the length of the clavicular fibres of the muscle, just behind the fold of its sternal part. Usually the upper electrode was just below and behind the angle of the jaw and the lower electrode about 3 cm. above the clavicle. Preliminary experiments showed that there was no significant difference in activity between the sternal and clavicular heads.

\* Part of a thesis accepted for the Doctorate of Philosophy by the University of London.

*The scaleni.* A pair of electrodes was placed 2-3 cm. apart in the lower anterior angle of the posterior triangle, just behind the clavicular fibres of the sternomastoid; the lower electrode was placed immediately above the clavicle. In this site the electrodes are over the scalenus medius and probably the lower fibres of the levator scapulae.

## RESULTS

### *General Remarks*

The main study was made with the subjects supine, as this was the only posture in which complete relaxation could be easily obtained. It is difficult to detect respiratory variation in the electromyogram against a background of postural activity. Even in the supine posture there was often considerable continuous activity unless care was taken to place and support the head in a comfortable position. The insertion of a mouthpiece also tended to cause continuous activity which increased when it was held firmly in the mouth, as when static inspiratory and expiratory efforts were being made.

The possible contribution of the platysma to the activity recorded at either of the sites examined was considered. The electrodes over the sternomastoid also lie over the platysma; as little respiratory activity is recorded in this situation the role of the platysma is insignificant. The greater activity commonly recorded over the scaleni may thus be attributed to these muscles and not to the platysma.

### *Skeletal movements*

The slightest attempt by the subject to raise his head from the couch caused marked activity in both muscles. Elevation of the shoulder caused marked activity which was detected by the electrodes over the scaleni but not by those over the sternomastoid.

### *Observations in the supine position*

#### *Quiet breathing*

*Sternomastoid.* There was no detectable activity in any of the subjects when they were relaxed and comfortable.

*Scaleni.* In three subjects there was no detectable activity. In the fourth there was continuous activity like that described above which could not be abolished by adjusting the position of the head and which showed no respiratory rhythm; he was examined again 6 months later with the same result. In the fifth subject there was considerable spontaneous activity; after reducing it as much as possible by attention to posture and comfort a respiratory rhythm persisted (Fig. 1).

### *Maximum inspiration and expiration*

There was marked activity at both electrode sites in all subjects on maximum inspiration. On maximum expiration there was either no activity or only slight activity.

*Graded inspiratory efforts performed at the resting respiratory level*

There was a clearly discernible gradation in the intensity of the muscular activity over a range of pressures from  $-10$  to  $-50$  cm.  $H_2O$  (i.e. intra-pulmonary pressures  $10-50$  cm.  $H_2O$  below atmospheric). This gradation was obvious on simple inspection of the records.

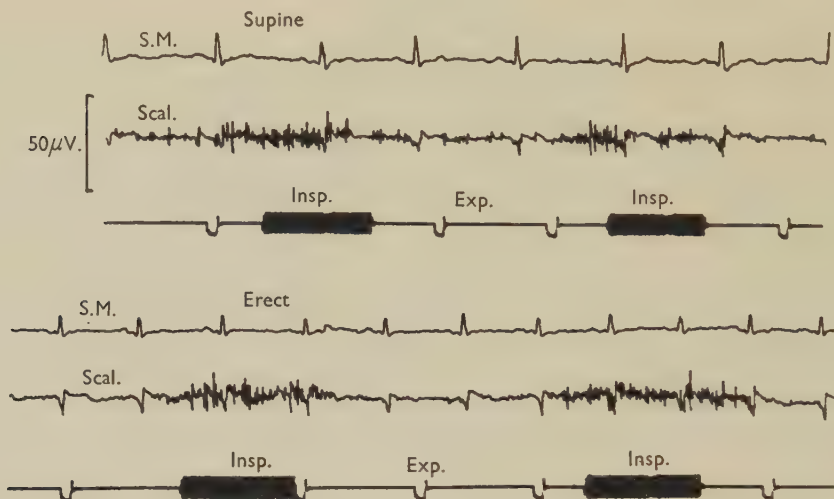


Fig. 1. Quiet breathing. Subject R. B. Electromyograms recorded from the sternomastoid (S.M.) and scalene muscles (Scal.). The phases of respiration were signalled by an observer. The onset of inspiration precedes the beginning of the signal. Time in sec. Regular deflexions represent the electrocardiogram. No activity in sternomastoids. Activity of scaleni present in both supine and erect posture. There is marked increase in activity with inspiration. Compare Fig. 3.

*Graded expiratory efforts performed at the resting respiratory level*

*Sternomastoid.* In three of the subjects slight muscular activity developed on making expiratory efforts in which the intra-pulmonary pressure rose to  $+40$  cm.  $H_2O$ . The intensity of the activity was not related to that of the effort. In the other two subjects the presence of variable spontaneous activity prevented any conclusions being made.

*Scaleni.* During expiratory efforts resulting in intra-pulmonary pressures of  $+20$  to  $30$  cm.  $H_2O$  spontaneous activity if present was suppressed. In no subject did fresh activity appear at these pressures. However, during efforts of  $+40$  cm.  $H_2O$  and above, all subjects showed definite or marked activity (Fig. 2).

*Increased pulmonary ventilation (Fig. 3)*

The subjects rebreathed expired air from the spirometer with no  $CO_2$  absorber in the circuit. As a result the ventilation rate increased to  $50-80$  l./min.; at this stage the subjects became too distressed to continue. As they became used to this procedure they were encouraged to increase the volume of their breathing voluntarily



and they were then able to produce a steadily progressive increase to well over 100 l./min.

*Sternomastoid.* Activity towards the end of the phase of inspiration occurred in four of the subjects at minute volumes of 61.5, 75, 105, 41 l. respectively. In the fifth subject continuous activity appeared at 97.5 l./min. It is obvious on anatomical grounds that the tidal volume may be more important than the ventilation rate in determining the recruitment of the sternomastoid. The data are therefore given

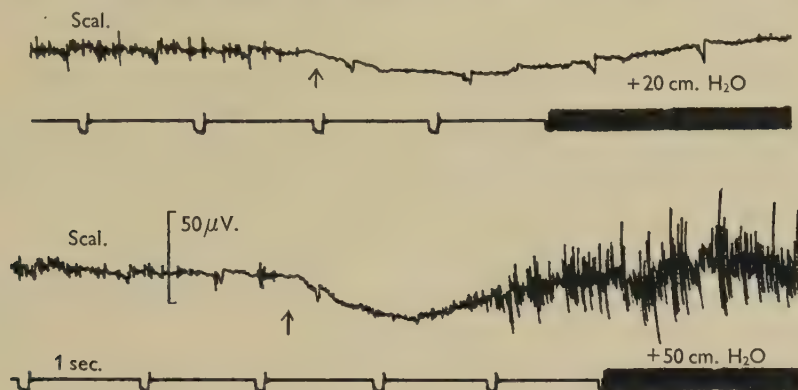


Fig. 2. Subject R. B., supine. Scal., the electromyogram recorded from the scalene muscles. Time in sec. The subject was breathing through a mouthpiece. At the arrow he began to make an expiratory effort. The signal marks the period of maintenance of the expiratory pressure indicated. This subject showed continuous irregular activity in the scalene muscles, as seen at the beginning of each record. An expiratory effort of +20 cm. H<sub>2</sub>O (intra-pulmonary pressure 20 cm. H<sub>2</sub>O above atmospheric) was associated with a decrease in background activity; an effort of +50 cm. H<sub>2</sub>O was associated with a marked increase (see discussion).

Table 1. *Sternomastoid activity during increased pulmonary ventilation: supine posture*

The respiratory rate per minute, tidal volume (T.V.) in litres and minute volume (M.V.) in litres per min. at which activity appeared are given for each subject. In the fifth and sixth columns the critical tidal volume (at which activity appeared) is expressed as a percentage of the subject's vital capacity (v.c.) and inspiratory capacity (Insp. capacity).

Subject	Critical values			Critical tidal volume as percentage of	
	Rate	T.V.	M.V.	v.c.	Insp. capacity
G. M.	22.5	2.7	61.5	60	80
G. Ho.	28.5	2.6	75	65	80
J. Da.	36	2.9	105	60-65	70-75
R. B.	30	3.2	97	55	70-75
G. Ha.	14	2.9	41	65-70	80-85

in more detail in Table 1; the tidal volume at which activity first appeared is also given as a percentage of the vital capacity and the inspiratory capacity. Three conclusions can be drawn from Table 1:

(i) At least 70 % of the inspiratory capacity can be used as tidal volume by the normal subject without using the sternomastoid as a muscle of inspiration.

(ii) If the rate of breathing is increased to 30 per min. (as it was in subjects J. Da.

and R. B.) the maximum exercise level of ventilation can be attained without using the sternomastoid.

(iii) If 10 cm.  $H_2O/l.$  is assumed to be the elastance of the chest wall and lungs, all the subjects were able to exert a force equivalent to an inspiratory pressure of 20 cm.  $H_2O$  below atmospheric without using the sternomastoid. (The data of Rahn, Otis, Chadwick & Fenn (1946) give a value of about 14 cm.  $H_2O/l.$  for the elastance). The

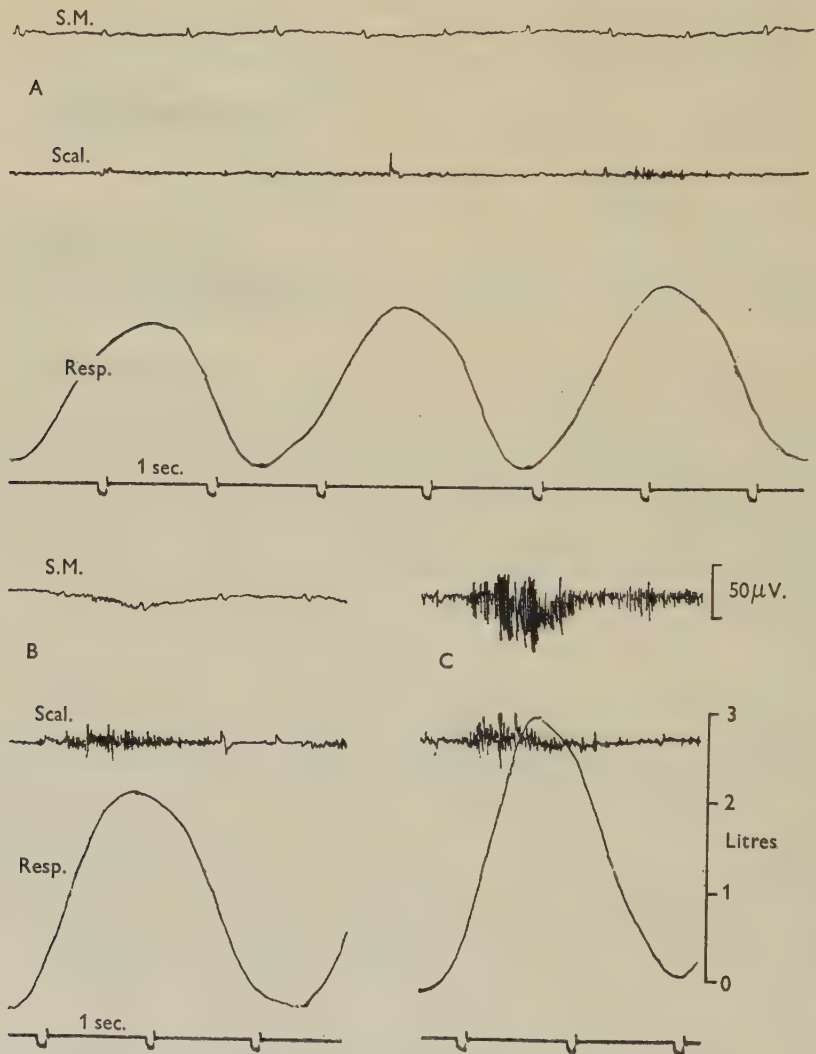


Fig. 3. Subject G. M., supine. Electromyograms recorded from the sternomastoid (S.M.) and scalene (Scal.) muscles. Resp. = the record of respiration (inspiration upwards). Time in sec. The subject re-breathed expired air from a 6 l. spirometer. A shows the onset of inspiratory activity in the scaleni. B and C show the development of activity in the sternomastoid. At the beginning of A the ventilation rate was 42 l./min. and at the end it was 52 l./min. In B the ventilation rate was 60 l./min. and, in C, 78 l./min.

muscular activity recorded during static voluntary inspiratory efforts of  $-10$  cm.  $H_2O$  is difficult to account for. The explanation may be that the accessory muscles are always more readily employed in voluntary inspiratory efforts, possibly because the ordinary muscles such as the diaphragm and intercostals are not subject to sufficiently exact voluntary control.

*Scaleni.* Table 2 sets out the rate and depth of breathing at which activity appeared in the three subjects who showed no activity (spontaneous or rhythmic) during quiet breathing. It is clear that activity develops earlier in the scaleni.

Table 2. *Activity of scaleni during increased pulmonary ventilation*

The respiratory rate per minute, tidal volume (t.v.) in litres and minute volume (m.v.) in litres per min. at which activity appeared are given for three subjects. In the fifth and sixth columns the critical tidal volume (at which activity appeared) is expressed as a percentage of the subject's vital capacity (v.c.) and inspiratory capacity (Insp. capacity).

Subject	Critical values			Critical tidal volume as percentage of	
	Rate	T.V.	M.V.	v.c.	Insp. capacity
G. M.	27	1.6	42	30	40-45
J. Da.	26	2.6	68	55-60	65
G. Ha.	15	2.1	31.5	45-50	60

No definite expiratory activity was detected in either muscle in any subject until the ventilation rate was so great that the significance of the activity was doubtful. This is because severe efforts or general distress often cause widespread slight contraction of many muscles, some of which are not associated with the movement which the subject is performing.

#### *The effects of posture*

In four subjects (one of whom was not included in the series of experiments described above) records were taken of quiet breathing and increased pulmonary ventilation in the erect posture.

*Sternomastoid.* Although there was a tendency for irregular activity to appear in the erect posture it could always be abolished, and no subject showed constant rhythmic activity. Table 3 gives the rate and depth of breathing at which activity

Table 3. *Sternomastoid activity during increased pulmonary ventilation: erect posture*

This table gives the tidal volume (t.v.) in litres, respiratory rate per minute and minute volume (m.v.) in litres per min. at which activity appeared in the sternomastoid muscle during progressively increasing pulmonary ventilation in the erect posture.

Subject	T.V.	Rate	Minute volume
R. B.	1.4	22	31
M. Ch.	2.1	24	51
G. M.	2.7	27	73
G. Ha.	1.9	25	48

appeared during increased pulmonary ventilation. The data suggest that the sternomastoid is more readily used in the erect than in the supine posture, but too much emphasis must not be placed on the values recorded. They probably underestimate



the threshold of recruitment because it is difficult, even for trained subjects, in the erect posture with a mouthpiece, to relax and allow their breathing to be increased by CO<sub>2</sub> accumulation. Previous experience with subjects R. B. and M. Ch. suggests that they are both capable of greater minute volumes than G. M. before using their accessory muscles of inspiration.

*Scaleni.* In two subjects there was well-marked rhythmic inspiratory activity in the erect posture. In the third subject there was considerable irregular activity which with adjustment of the posture of the shoulders showed slight rhythmicity. In the fourth subject (G. M.) there was either no activity or slight irregular activity which became rhythmic at a minute volume of 50 l./min. ( $27 \times 1.9$  l.).

#### DISCUSSION

*Scalene muscles.* On anatomical grounds the three scaleni would appear to have the same fundamental actions. The scalenus medius was probably the main contributor to the records obtained from the electrode site used in this study. It is possible that the levator scapulae or the omo-hyoid also contributed to the electromyogram, but it is unlikely that the considerable respiratory activity recorded arose in these muscles. To settle this point it would have been desirable to explore the region with needle electrodes. This procedure was considered to be unjustifiable because of the risks of damaging the major nerves and blood vessels present in this region of the neck.

The importance of the scaleni as muscles of inspiration is variously assessed by different authorities. Some regard them as ordinary muscles of inspiration equal in importance to the intercostals. Others class them as accessory muscles of inspiration equal in importance to the sternomastoid. The findings in the present study show that in fact the scaleni may be active in normal subjects even during quiet breathing, but that the sternomastoid is only employed at very high levels of ventilation. Weddell, Feinstein & Pattle (1944) observed inspiratory contraction of the scaleni during quiet breathing. Schill (1942) observed by palpation that in patients with heart or lung disease the scaleni are used at an earlier stage of dyspnoea than the sternomastoids.

These observations, however, do not establish the importance of the scaleni in pulmonary ventilation. Duchenne (1867) observed that costosuperior breathing continued in a patient who had lost most of his scaleni. Joly & Vincent (1937) found that scalenotomy performed in patients with pulmonary tuberculosis did not reduce the amplitude of the rib movements in breaths of normal depth (whereas paralysis of the intercostals did). Giauni (1936) observed that although scalenotomy caused an immediate decrease in vital capacity, considerable recovery occurred later and in some cases there was a complete return to normal. R. Fick (1923) calculated that the scaleni are only potentially one fifth as important as the intercostals.

The findings of considerable activity during moderately severe voluntary expiratory efforts (Fig. 2) suggests the possibility that the scaleni may be of importance in these circumstances and in such procedures as coughing or straining. Their action under these conditions may be to fix the upper ribs and prevent the thoracic cage from being pulled downwards by the abdominal muscles, or it may be

to provide support for the apex of the lung to prevent it from bulging upwards. Recordings taken during coughing and straining did in fact show considerable activity, but electromyograms taken at any site in the trunk show activity during these procedures. The technique of graded voluntary efforts was used to distinguish muscular contraction of importance from the minor generalized activity which occurs in violent efforts.

*Sternomastoid muscles.* The importance of the sternomastoids in dyspnoea is a commonplace clinical observation. The present study shows how surprisingly slight is their respiratory activity in normal subjects. It appears that they become important when the respiratory level is elevated and the ordinary muscles of inspiration are operating at much reduced mechanical advantage. Cournand, Brock, Rappaport & Richards (1936) suggested that a spastic state of the accessory muscles of inspiration might be a contributory cause of dyspnoea in patients with pulmonary fibrosis. This suggestion was based on the observation that some patients who are unduly dyspnoeic in relation to the assessment of their ventilatory function and the tension of their blood gases, show over-activity of these muscles.

Duchenne recounted the case of a young man with a high cervical transection of the spinal cord who breathed for some weeks apparently by means of his sternomastoids alone. He was very cyanosed and when he was given artificial respiration contraction of the sternomastoids ceased. On stopping the artificial respiration the contraction only returned when he again became very cyanosed.

#### SUMMARY

1. The respiratory activity of the scalene and sternomastoid muscles has been examined electromyographically in five healthy young men.

2. The scaleni were readily employed as muscles of inspiration and in some of the subjects they were active during quiet breathing, especially in the erect posture.

3. The scaleni also showed considerable activity during moderately severe expiratory efforts. They may be of importance in such actions as coughing or straining.

4. The sternomastoids only showed activity during very deep breaths, and all the subjects were able to attain high ventilation rates without using them.

I am grateful to Prof. Samson Wright and to Dr P. H. S. Silver for their guidance and advice. I would like to thank Prof. E. W. Walls for reading the typescript.

#### REFERENCES

- CAMPBELL, E. J. M. & GREEN, J. H. (1953). The expiratory function of the abdominal muscles in man. An electromyographic study. *J. Physiol.* **120**, 409-418.
- COURNAND, A., BROCK, H. J., RAPPAPORT, I. & RICHARDS, D. W. (1936). Disturbance of action of respiratory muscles as a contributing cause of dyspnea. *Arch. intern. Med.* **57**, 1008-1026.
- DUCHENNE, G. B. A. (1867). *Physiologie des mouvements démontrée à l'aide de l'expérimentation électrique et de l'observation clinique, et applicable à l'étude des paralysies et des déformations*. Translated by Kaplan, E. B. (1949), pp. 443-503. Philadelphia: Lippincott.
- FICK, R. (1923). Über die Zwischenrippenmuskeln. *S.B. preuss. Akad. Wiss. (Physik.-math. Kl.)*, pp. 65-72.

- GIAUNI, G. (1936). Esplorazione pneumografica della cinematica toracica e della capacità vitale dopo scalenotomia e dopo scalenofrenico-exeresi. *Clin. med. ital.* **67**, 783-796.
- JOLY, H. & VINCENT, PH.-A. (1937). Rôle respiratoire des muscles scalènes et inter-costaux étudié en fonction de la collapsio-thérapie pulmonaire. *Arch. méd.-chir. Appar. resp.* **12**, 392-404.
- RAHN, H., OTIS, A. B., CHADWICK, L. E. & FENN, W. O. (1946). The pressure-volume diagram of the thorax and lung. *Amer. J. Physiol.* **146**, 161-178.
- SCHILL, E. (1942). Über die Hilfsmuskeln der Einatmung. *Beitr. klin. Tuberk.* **98**, 380-381.
- WEDDELL, G., FEINSTEIN, B. & PATTLE, R. E. (1944). The electrical activity of voluntary muscle in man under normal and pathological conditions. *Brain*, **67**, 178-257.



## THE BRONCHO-PULMONARY SEGMENTS IN THE SHEEP\*

By W. C. D. HARE

*Department of Anatomy, Royal (Dick) School of Veterinary Studies,  
University of Edinburgh†*

### INTRODUCTION

The idea that there were limited areas within a pulmonary lobe was first formulated some time ago. Ewart (1889) was of the opinion that the collateral branches of the lobar bronchi of the right and left lungs were each responsible for the ventilation of a definitely limited part of the lobe concerned.

About forty years later, Kramer & Glass (1932) were among the first to attempt to define these areas, which were given the name of lung segments. They stated that: 'This unit, the broncho-pulmonary segment, is a subdivision of a pulmonary lobe and represents not only an anatomic but also a pathologic unit. Each segment occupies a definite constant position in the pulmonary architecture and thoracic cavity and is supplied by a constantly placed bronchus whose orifice is situated in a large lobar bronchus and is easily visible to the bronchoscopist.' Lucien & Weber (1936), when referring to similar subdivisions, described them as 'territories of ventilation'.

The segmental anatomy in the human lung has been further investigated by a number of workers, amongst whom Foster-Carter (1942), Jackson & Huber (1943), Brock (1946) and Rap (1947) have given detailed descriptions of the bronchial tree with reference to the broncho-pulmonary segments and their boundaries. The researches of the above workers emphasized the variations which occur in the broncho-pulmonary segments and the confusion which existed with regard to the nomenclature.

In recent years, the variations found in the broncho-pulmonary segments of the human lung have received investigation by several American workers. Boyden & Hartmann (1946) and Scannell (1947) carried out an analysis on the left upper lobe, Scannell & Boyden (1948) carried out an analysis on the right upper lobe, while Berg, Boyden & Smith (1949) and Smith & Boyden (1949) analysed the variations in the left lower lobe and right lower lobe respectively.

When, in 1949, the International Congress of Oto-Rhino-Laryngology was held in London, the opportunity was taken to hold a meeting to discuss the nomenclature of the bronchial tree and the segments. A basic international nomenclature was recommended, and this was accepted later by the Thoracic Society and recorded in *Thorax* (1950).

Bressou & Vladutiu (1939) carried out a survey of seventy-two lungs belonging to various species of domestic animals, including ten lungs of sheep, with a view to

\* Based on part of a thesis accepted for the Ph.D. degree of the University of Edinburgh in December 1953.

† Present address, Department of Anatomy, Ontario Veterinary College, Guelph, Ontario, Canada.

analysing the branching of the bronchial tree and to dividing the lung up into a number of autonomous zones, but apart from this, as far as is known, no specific work has been carried out on the segmental anatomy of the lungs of the sheep.

#### MATERIAL AND METHODS

Following an analysis of the main branches of the bronchial tree in a number of lungs by means of dissections and corrosion casts, the broncho-pulmonary segments were studied in the lungs of twenty sheep, using three methods. These were:

(a) *Air inflation.* This is a quick, temporary method which was used to demarcate the segments in the lungs of six adult sheep. Each segment was inflated with air passed under a pressure sufficient to produce demarcation, but not to break down the alveoli.

(b) *Injection of gelatin solutions.* This is a permanent method of defining the segments which was used in seven pairs of lungs freshly isolated from adult sheep. The conductory and respiratory parts of the bronchial tree of each segment were filled under pressure with a 15 % coloured solution of Gurr's bacteriological gelatin in water, which was contained in an 8 oz. metal bladder syringe.

(c) *Fine corrosion casts of the bronchial tree.* This, also, is a permanent method of defining the segments of the lungs. The conductory and respiratory parts of the bronchial tree in the lungs of lambs 3-4 days old were filled with an injection mass. The lungs were injected while *in situ* to prevent the collapse and loss of shape which ensued if they were removed from the thorax. The mass used was Marco Resin 26c\* with chalk added as a filler, and it was made up as follows: 500 ml. solution A, 70 ml. solution B, 70 ml. solution C (plasticiser), and 8 oz. chalk.

When the mass had hardened, the lungs were removed from the thorax and placed in a tank containing a strong solution of sodium sulphide. They were then incubated at 45° C., until the lung tissue had been corroded leaving a cast of the bronchial tree. After the cast had been dried, deep fissures appeared along the lines of the inter-segmental connective tissue planes and allowed the segments to be identified.

#### DESCRIPTION

##### *Lobes*

The lungs are the paired respiratory organs which occupy a large part of the thoracic cavity. Each lung is subdivided by fissures or interlobar connective tissue planes into lobes, and each lobe is ventilated by a large bronchus that arises either from a main bronchus or from the trachea.

The right lung is considerably larger than the left and consists of four lobes: an apical, a cardiac, an intermediate and a diaphragmatic.

The apical lobe is large and consists of two distinct parts, a cranial and a caudal, which are incompletely separated from each other by an indentation in the ventral border, the cardiac notch (Pl. 1, fig. 1).

The cardiac lobe is tongue-shaped and quadrilateral in cross-section. It is separated from the apical lobe by a fissure at the level of the fourth and fifth ribs,

\* Supplied by Cromwell and Co. Ltd., Bishop's Stortford, Herts.

and from the diaphragmatic lobe by a fissure at the level of the fifth and sixth ribs (Pl. 1, fig. 1).

The intermediate lobe is cone-shaped, with a concave base which rests on the diaphragm; its apex is attached to the mediastinal aspect of the lung just caudal to the hilus. In the dorsal part of its lateral surface, the intermediate lobe presents a groove for the caudal vena cava and the right phrenic nerve (Pl. 1, fig. 3).

The diaphragmatic lobe has the form of a three-sided pyramid; its concave base, or diaphragmatic surface, is bounded by the basal border (Pl. 1, figs. 1, 3).

The left lung consists of two lobes, an apico-cardiac and a diaphragmatic.

The apico-cardiac lobe is separated from the diaphragmatic lobe by a fissure at the level of the fifth and sixth ribs. It presents a small, pointed, apical part, which forms the cranial dorsal part of the lobe, and a narrow, tongue-shaped cardiac part, which forms the caudal ventral part of the lobe (Pl. 1, fig. 2).

The diaphragmatic lobe resembles the corresponding lobe in the right lung (Pl. 1, fig. 2).

### *Bronchial tree*

A knowledge of the main branches of the bronchial tree is indispensable for an understanding of the segmental anatomy of the lungs. Although numerous variations occur in the ramifications of the bronchial tree, it is possible to recognize a number of large bronchi which are constantly present.

At the level of the third rib, the trachea gives off the right apical lobar bronchus from its right, ventral lateral aspect and then, at the level of the fifth rib, it bifurcates into the right and left main bronchi, which ventilate the right lung (with the exception of the apical lobe) and the left lung respectively.

Approximately 15 mm. below the bifurcation of the trachea, the right main bronchus gives rise to two bronchi from a common opening in its ventral aspect. One bronchus passes in a ventral and lateral direction to ventilate the cardiac lobe, while the other bronchus runs in a ventral, medial and caudal direction to ventilate the intermediate lobe. Caudal to the origin of these bronchi, the continuation of the right main bronchus is known as the right diaphragmatic lobar bronchus (Text-fig. 1).

The left main bronchus gives off the apico-cardiac lobar branches from its ventral lateral aspect, approximately 16 mm. below the bifurcation of the trachea, and is continued as the left diaphragmatic lobar bronchus (Fig. 1).

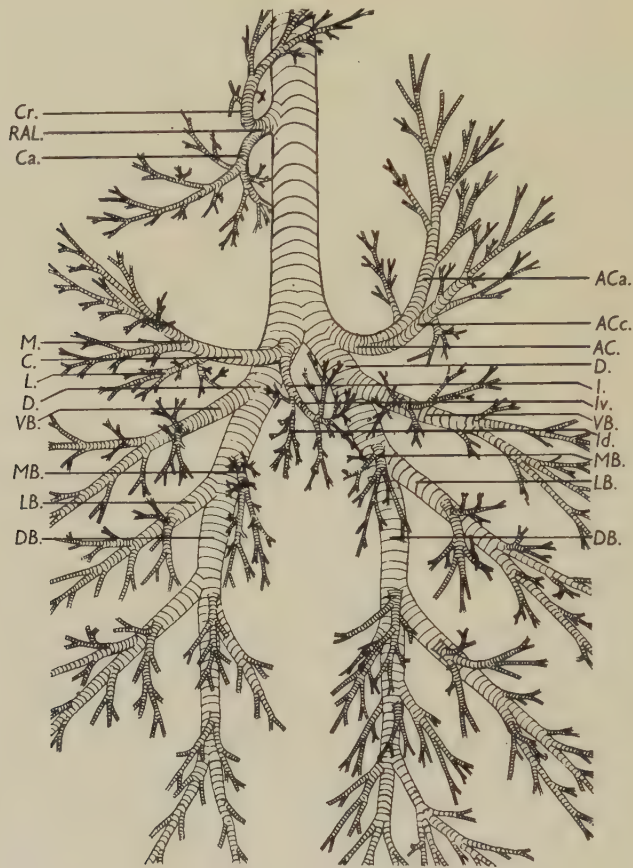
Arising from the lobar bronchi there are large bronchi which ventilate the large independent areas of a lobe defined by Kramer & Glass (1932) as broncho-pulmonary segments, and these are called segmental bronchi.

The right apical lobar bronchus runs in a ventral, lateral and caudal direction for approximately 10 mm. before dividing into two bronchi, the cranial and caudal segmental bronchi. The cranial bronchus passes in a cranial, ventral and lateral direction to ventilate the cranial segment of the lobe, and the caudal bronchus runs in a caudal, ventral and lateral direction to ventilate the caudal segment of the lobe (Text-fig. 2).

The right cardiac lobar bronchus gives off a lateral segmental bronchus from its dorsal lateral aspect, about 11 mm. below its origin from the right main bronchus. This bronchus passes in a lateral direction to ventilate the lateral broncho-

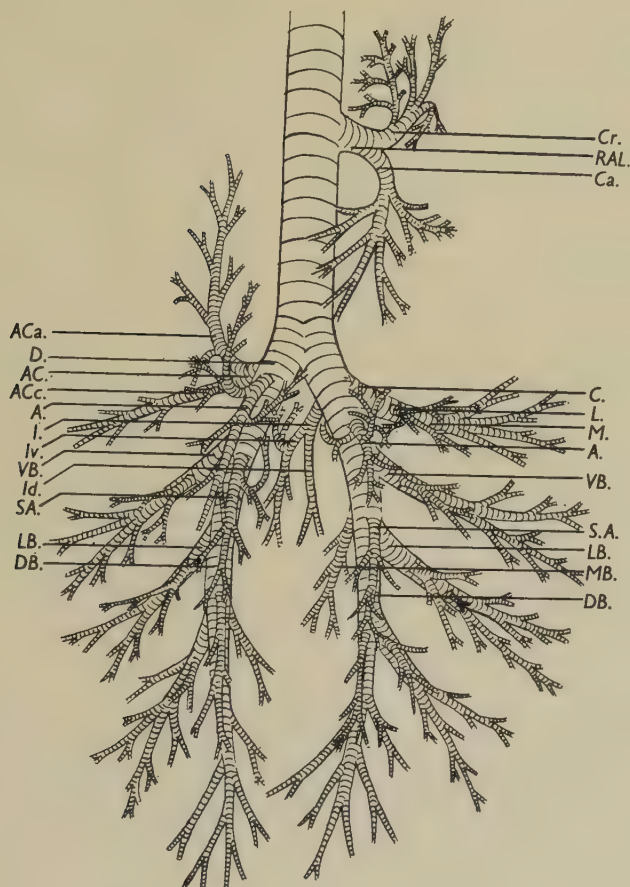


pulmonary segment of the cardiac lobe. The continuation of the lobar bronchus ventilates the medial broncho-pulmonary segment of the lobe and is known as the medial segmental bronchus (Text-figs. 1, 2).



Text-fig. 1. Ventral view of the bronchial tree.

- |             |  |
|-------------|--|
| <i>RAL.</i> | Right apical lobar bronchus                                |
| <i>Cr.</i>  | Cranial segmental bronchus of the right apical lobe        |
| <i>Ca.</i>  | Caudal segmental bronchus of the right apical lobe         |
| <i>D.</i>   | Diaphragmatic lobar bronchus                               |
| <i>C.</i>   | Right cardiac lobar bronchus                               |
| <i>L.</i>   | Lateral segmental bronchus of the right cardiac lobe       |
| <i>M.</i>   | Medial segmental bronchus of the right cardiac lobe        |
| <i>I.</i>   | Right intermediate lobar bronchus                          |
| <i>Id.</i>  | Dorsal segmental bronchus of the right intermediate lobe   |
| <i>Iv.</i>  | Ventral segmental bronchus of the right intermediate lobe  |
| <i>AC.</i>  | Apico-cardiac lobar bronchus                               |
| <i>ACa.</i> | Apical segmental bronchus of the apico-cardiac lobe        |
| <i>ACc.</i> | Cardiac segmental bronchus of the apico-cardiac lobe       |
| <i>VB.</i>  | Ventral basal segmental bronchus of the diaphragmatic lobe |
| <i>MB.</i>  | Medial basal segmental bronchus of the diaphragmatic lobe  |
| <i>LB.</i>  | Lateral basal segmental bronchus of the diaphragmatic lobe |
| <i>DB.</i>  | Dorsal basal segmental bronchus of the diaphragmatic lobe  |



Text-fig. 2. Dorsal view of the bronchial tree.

- |             |  |
|-------------|--|
| <i>RAL.</i> | Right apical lobar bronchus                                |
| <i>Cr.</i>  | Cranial segmental bronchus of the right apical lobe        |
| <i>Ca.</i>  | Caudal segmental bronchus of the right apical lobe         |
| <i>D.</i>   | Diaphragmatic lobar bronchus                               |
| <i>C.</i>   | Right cardiac lobar bronchus                               |
| <i>L.</i>   | Lateral segmental bronchus of the right cardiac lobe       |
| <i>M.</i>   | Medial segmental bronchus of the right cardiac lobe        |
| <i>AC.</i>  | Apico-cardiac lobar bronchus                               |
| <i>ACa.</i> | Apical segmental bronchus of the apico-cardiac lobe        |
| <i>ACc.</i> | Cardiac segmental bronchus of the apico-cardiac lobe       |
| <i>A.</i>   | Apical segmental bronchus of the diaphragmatic lobe        |
| <i>VB.</i>  | Ventral basal segmental bronchus of the diaphragmatic lobe |
| <i>SA.</i>  | Subapical segmental bronchus of the diaphragmatic lobe     |
| <i>LB.</i>  | Lateral basal segmental bronchus of the diaphragmatic lobe |
| <i>MB.</i>  | Medial basal segmental bronchus of the diaphragmatic lobe  |
| <i>DB.</i>  | Dorsal basal segmental bronchus of the diaphragmatic lobe  |
| <i>I.</i>   | Right intermediate lobar bronchus                          |
| <i>Id.</i>  | Dorsal segmental bronchus of the right intermediate lobe   |
| <i>Iv.</i>  | Ventral segmental bronchus of the right intermediate lobe  |

The intermediate lobar bronchus generally gives off a segmental bronchus from its caudal aspect, approximately 10 mm. from its origin. This bronchus, which runs in a caudal direction, ventilates the dorsal broncho-pulmonary segment of the intermediate lobe and is known as the dorsal segmental bronchus. The continuation of the lobar bronchus ventilates the ventral broncho-pulmonary segment of the lobe and is known as the ventral segmental bronchus (Text-figs. 1, 2).

The right diaphragmatic lobar bronchus gives rise to a number of segmental bronchi which are constantly present, although the order in which they arise may vary considerably. The ventral basal and lateral basal segmental bronchi arise from its ventral lateral aspect and run in a ventral, lateral and slightly caudal direction to ventilate the ventral basal and lateral basal segments. The apical and subapical segmental bronchi arise from its dorsal aspect and run in a caudal and dorsal direction to ventilate the apical and subapical segments. The medial basal segmental bronchus, which ventilates the medial basal segment, arises from the ventral or ventral medial aspect of the lobar bronchus and runs in a ventral, medial and caudal direction. Caudal to the level at which these bronchi arise, the lobar bronchus is known as the dorsal basal segmental bronchus (Text-figs. 1, 2).

The apico-cardiac lobar bronchus runs in a ventral, lateral and slightly caudal direction for approximately 10 mm., and then divides into two bronchi: an apical segmental bronchus which runs in a cranial direction to ventilate the apical broncho-pulmonary segment of the apico-cardiac lobe, and a cardiac segmental bronchus which runs in a ventral, lateral and slightly caudal direction to ventilate the cardiac broncho-pulmonary segment of the lobe.

The left diaphragmatic lobar bronchus gives off a series of segmental bronchi, similar to those given off by the right diaphragmatic lobar bronchus, but with a more constant order of origin; it is then continued as the dorsal basal segmental bronchus. In some lungs, the medial basal broncho-pulmonary segment is absent and the area is ventilated instead by a bronchus arising from the lateral basal segmental bronchus.

#### *Broncho-pulmonary segments*

In this description of the broncho-pulmonary segments in the sheep, an attempt has been made to adopt a scheme and nomenclature similar to the international nomenclature accepted by the Thoracic Society for the human lung. The nomenclature adopted for the human lung is found to be unsuitable when applied to certain segments in the sheep, which is consistent with what one would expect when a nomenclature based on a biped is applied to a quadruped, and in the present paper has been modified accordingly to suit the quadruped.

Generally speaking, each broncho-pulmonary segment may be described as being wedge-shaped, with the apex of the wedge directed towards the origin of the segmental bronchus and the base forming part of the surface of the lung. Lying between the segments there are planes of connective tissue, the intersegmental planes, and by breaking down these planes it is possible to separate the segments.

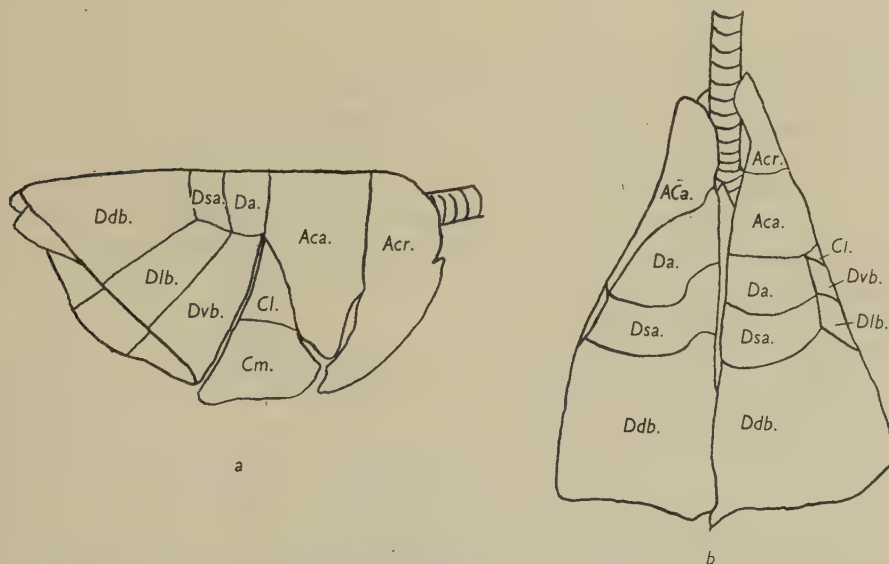
As mention has already been made of the lobation of the lungs, it is convenient to describe the segments under the heading of the lobe of which they form a part.

*The right apical lobe.* The segments of this lobe differ considerably from those of



the right upper lobe in the human. The lobar bronchus divides into two segmental bronchi which ventilate the cranial and caudal segments of the lobe.

The cranial broncho-pulmonary segment forms the cranial part of this lobe, which occupies the cranial part of the thoracic cavity; its caudal boundary is formed by the intersegmental plane that lies between the cranial and caudal parts of the lobe, and is marked on the external surface by a line drawn in a dorsal direction from the dorsal part of the cardiac notch (Text-fig. 3*a*).



Text-fig. 3. The broncho-pulmonary segments. (*a*) Lateral view of the right lung. (*b*) Dorsal view of the right and left lungs.

- Acr.* Cranial segment of the right apical lobe
- Aca.* Caudal segment of the right apical lobe
- Cl.* Lateral segment of the right cardiac lobe
- Cm.* Medial segment of the right cardiac lobe
- ACa.* Apical segment of the apico-cardiac lobe
- Dvb.* Ventral basal segment of the diaphragmatic lobe
- Dlb.* Lateral basal segment of the diaphragmatic lobe
- Ddb.* Dorsal basal segment of the diaphragmatic lobe
- Da.* Apical segment of the diaphragmatic lobe
- Dsa.* Subapical segment of the diaphragmatic lobe

The caudal broncho-pulmonary segment forms the caudal part of the lobe. Its caudal boundary is indicated, ventrally, by the fissure that lies between the apical and cardiac lobes and, dorsally, by the intersegmental plane that lies between the apical and diaphragmatic lobes; this plane is marked on the surface by a line drawn in a dorsal direction from the dorsal part of the cardiac lobe over the vertebral border of the lung and continued ventrally to the right main bronchus (Text-figs. 3*a*, *b*, 5*a*).

*The right cardiac lobe.* As in the middle lobe of the human lung, this lobe is considered to be composed of a lateral and a medial broncho-pulmonary segment.

The lateral segment is ventilated by the lateral segmental bronchus, and forms the dorsal lateral part of the lobe (Text-fig. 3a).

The medial broncho-pulmonary segment is ventilated by the continuation of the lobar bronchus, the medial segmental bronchus, and forms the remaining part of the lobe (Text-fig. 3a).

The intersegmental plane separating these two segments is marked on the lateral surface by a line drawn transverse to the long axis and about half-way along the length of the lobe. From this external demarcation, the plane extends in a dorsal and medial direction.

*The intermediate lobe.* This lobe, which is absent in the human, is composed of two broncho-pulmonary segments, a dorsal and a ventral.

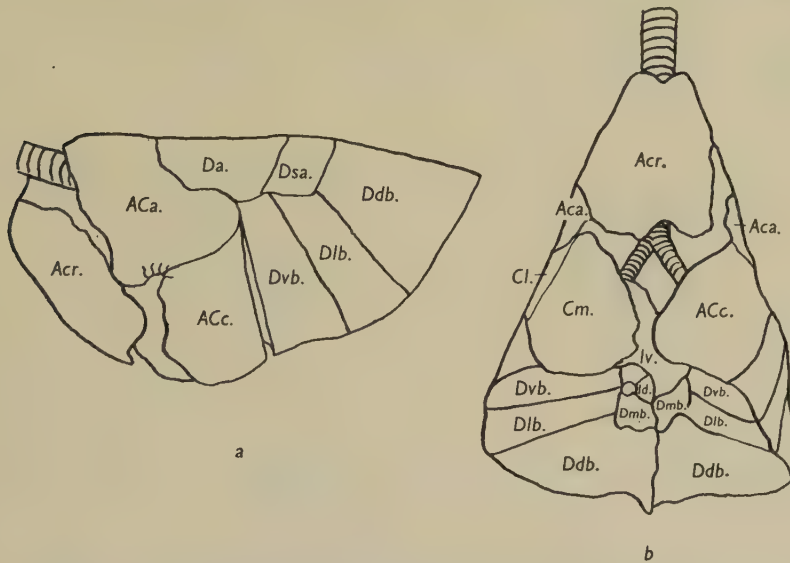
The dorsal broncho-pulmonary segment is ventilated by the dorsal segmental bronchus which arises from the caudal aspect of the lobar bronchus about 10 mm. from its origin, and the ventral broncho-pulmonary segment is ventilated by the continuation of the lobar bronchus, the ventral segmental bronchus. The connective tissue between the segments lies on a frontal plane in the medial wall of the caval foramen, and extends forward from the base of the lobe to the ventral segmental bronchus (Text-figs. 4b, 5a).

*The right diaphragmatic lobe.* This lobe is composed of six broncho-pulmonary segments named as follows: ventral basal, lateral basal, dorsal basal, apical, sub-apical and medial basal.

The ventral basal broncho-pulmonary segment forms the cranial, ventral lateral part of the lobe and corresponds to the anterior basal segment of the right lower lobe in the human. Its cranial boundary is formed by the fissure that lies between the diaphragmatic and cardiac lobes. Its caudal boundary is formed by the connective tissue plane that lies between it and the lateral basal segment; this intersegmental plane is marked, on the costal surface of the lobe, by a line which starts approximately one-third of the way along the length of the basal border and runs in a cranial and dorsal direction towards the dorsal border of the lobe; it extends through the lobe and is marked on the diaphragmatic surface by a line which starts one-third of the way along the length of the basal border and passes towards the caval foramen (Text-figs. 3a, 4b); near the lobar bronchus, it separates the ventral basal segment from the medial basal segment. The dorsal boundary of the ventral basal segment is formed by the plane which lies between it and the apical segment. This intersegmental plane is indicated on the costal surface of the lobe by a line drawn in a caudal direction on a frontal plane from the dorsal extremity of the cardiac lobe; from this external demarcation, the plane extends in a medial and ventral direction through the lobe towards the diaphragmatic lobar bronchus (Text-fig. 3a).

The lateral basal broncho-pulmonary segment forms the wedge-shaped area which occupies the middle, ventral lateral part of the diaphragmatic lobe between the ventral basal and dorsal basal segments; it corresponds to the lateral basal segment of the right lower lobe in the human. Its cranial boundary is formed by the connective tissue plane that lies between it and the ventral basal segment, and has already been described. Its caudal boundary is formed by the connective tissue plane that lies between it and the dorsal basal segment; this plane is marked on the costal surface of the lobe by a line which starts from the basal border approximately two-

thirds of the way along its length and runs in a cranial and dorsal direction; it extends through the lobe, and is marked on the diaphragmatic surface by a line running in a cranial and medial direction from the basal border (Text-figs. 3*a*, 4*b*). Dorsally, the lateral basal segment is separated from the apical and subapical segments by the connective tissue plane which is indicated on the costal surface of



Text-fig. 4. The broncho-pulmonary segments. (*a*) Lateral view of the left lung. (*b*) Ventral view of the right and left lungs.

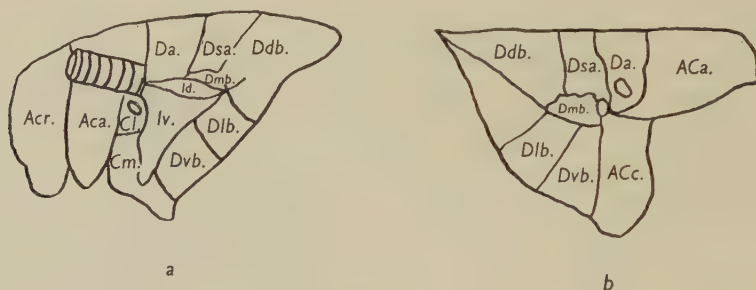
- Acr.* Cranial segment of the right apical lobe
- Aca.* Caudal segment of the right apical lobe
- ACa.* Apical segment of the apico-cardiac lobe
- ACc.* Cardiac segment of the apico-cardiac lobe
- Cl.* Lateral segment of the right cardiac lobe
- Cm.* Medial segment of the right cardiac lobe
- Iv.* Ventral segment of the right intermediate lobe
- Id.* Dorsal segment of the right intermediate lobe
- Dvb.* Ventral basal segment of the diaphragmatic lobe
- Dlb.* Lateral basal segment of the diaphragmatic lobe
- Ddb.* Dorsal basal segment of the diaphragmatic lobe
- Da.* Apical segment of the diaphragmatic lobe
- Dsa.* Subapical segment of the diaphragmatic lobe
- Dmb.* Medial basal segment of the diaphragmatic lobe

the lobe by a caudal extension of the line that marks the plane between the apical and ventral basal segments (Text-fig. 3*a*); from this external demarcation the plane extends through the lobe in a medial and ventral direction towards the diaphragmatic lobar bronchus. Medially, the lateral basal segment is separated by a connective tissue plane from the medial basal segment.

The apical broncho-pulmonary segment forms the cranial dorsal part of the lobe, and corresponds to the apical segment of the right lower lobe in the human. Its cranial boundary is formed by the intersegmental plane lying between it and the



caudal segment of the apical lobe, and has already been described. Its ventral lateral boundary is formed by the plane which lies between it, the lateral segment of the cardiac lobe, the ventral basal segment and the lateral basal segment, and has also been described above. The caudal boundary of the apical segment is formed by the plane lying between it and the subapical segment. This plane is marked on the surface of the lobe by a line drawn from the caudal limit of the ventral lateral boundary of the segment over the dorsal border to the mediastinal surface (Text-fig. 3*a*, *b*, 5*a*); from this external demarcation, it extends through the lobe in a cranial direction towards the diaphragmatic lobar bronchus.



Text-fig. 5. The broncho-pulmonary segments. (*a*) Medial view of the right lung.  
(*b*) Medial view of the left lung.

- Acr.* Cranial segment of the right apical lobe
- Aca.* Caudal segment of the right apical lobe
- Cl.* Lateral segment of the right cardiac lobe
- Cm.* Medial segment of the right cardiac lobe
- Iv.* Ventral segment of the right intermediate lobe
- Id.* Dorsal segment of the right intermediate lobe
- ACa.* Apical segment of the apico-cardiac lobe
- ACC.* Cardiac segment of the apico-cardiac lobe
- Da.* Apical segment of the diaphragmatic lobe
- Dsa.* Subapical segment of the diaphragmatic lobe
- Dvb.* Ventral basal segment of the diaphragmatic lobe
- Dlb.* Lateral basal segment of the diaphragmatic lobe
- Ddb.* Dorsal basal segment of the diaphragmatic lobe
- Dmb.* Medial basal segment of the diaphragmatic lobe

The subapical broncho-pulmonary segment forms the dorsal part of the diaphragmatic lobe which lies caudal to the apical segment and has no homologue in the human lung. The cranial boundary of this segment is formed by the connective tissue plane that lies between it and the apical segment, and has been described above. Caudally, the subapical segment is bounded by the connective tissue plane that lies between it and the dorsal segment; this plane is indicated on the surface of the lobe by a line drawn from the caudal limit of the ventral lateral boundary of the segment over the dorsal border to the mediastinal surface (Text-figs. 3*b*, 5*a*); from this external demarcation the plane extends through the lobe in a cranial direction towards the lobar bronchus. Medially, the subapical segment is separated from the medial basal segment by an intersegmental plane which is indicated on the

surface of the lung by a line lying on a frontal plane slightly ventral to the level of the lobar bronchus (Text-fig. 5*a*).

The medial basal broncho-pulmonary segment is a wedge-shaped part of the diaphragmatic surface. It is clearly not homologous with the medial basal segment in the human lung, which is ventilated by the first ventral or ventral medial bronchus and corresponds more closely to the intermediate lobe in the sheep. The medial basal segment is separated, laterally, from the ventral basal and lateral basal segments and, medially, from the subapical segment, by connective tissue planes which have already been described. Caudally, the segment is bounded by the intersegmental plane that lies between it and the dorsal basal segment; this plane is marked on the diaphragmatic surface by a line which runs laterally from the mediastinal aspect of the lobe towards the plane that lies between the medial basal and lateral basal segments (Text-fig. 4*b*); from this external demarcation it extends through the lung in a cranial and dorsal direction towards the lobar bronchus.

The dorsal basal broncho-pulmonary segment forms the caudal part of the diaphragmatic lobe, and is homologous with the posterior basal segment of the right lower lobe in the human. The segment is bounded cranially by the intersegmental planes, described above, which lie between it and the following segments: dorsally, the subapical segment; ventrally and laterally, the lateral basal segment; and ventrally, the medial basal segment (Text-figs. 3*a*, *b*, 4*b*).

*The apico-cardiac lobe.* This lobe is composed of two broncho-pulmonary segments, an apical and a cardiac, therefore the nomenclature used is not comparable with that adopted for the human lung, because, in the human, the left upper lobar bronchus is considered to divide into an upper division and a lingula (lower division) which, in turn, divide into segmental bronchi.

The apical broncho-pulmonary segment forms the cranial part of the left lung; it is separated by intersegmental planes from the cardiac segment caudally and ventrally, the ventral basal segment of the left diaphragmatic lobe caudally and the apical segment of the diaphragmatic lobe caudally and dorsally. The plane between it and the cardiac segment is indicated on the lateral surface of the lobe by a line drawn in either a caudal or a caudal dorsal direction from the cardiac notch to the line of the fissure, or the intersegmental plane, between the apico-cardiac and diaphragmatic lobes (Text-fig. 4*a*); from this external limit, the plane passes in a medial and slightly dorsal direction through the lobe towards the cardiac segmental bronchus. The intersegmental plane forming the dorsal and caudal boundary of the segment is usually marked on the surface of the lung by a line which starts on the dorsal border approximately at the level of the bifurcation of the trachea; from there it passes medially in a ventral direction towards the left main bronchus, and laterally in a caudal and ventral direction to meet the dorsal limit of the fissure or the intersegmental plane between the apico-cardiac and diaphragmatic lobes approximately 23 mm. ventral to the dorsal border of the lung. (Text-figs. 3*b*, 4*a*). From this external demarcation the plane passes through the lung towards the left main bronchus. The caudal boundary of the segment is formed by that part of the fissure or the intersegmental plane which separates the apico-cardiac and diaphragmatic lobes and lies between the planes described above (Text-fig. 4*a*).

The cardiac broncho-pulmonary segment forms the ventral lateral part of the

lobe; it is bounded, dorsally, by the intersegmental plane that lies between it and the apical segment and, caudally, by the fissure or the intersegmental plane, between it and the diaphragmatic lobe (Text-fig. 4a). The length of the fissure varies, and, if it is restricted, it is replaced by a plane of connective tissue which follows the course of the fissure.

*The left diaphragmatic lobe.* This lobe, like the right diaphragmatic lobe, is composed of six broncho-pulmonary segments named as follows: ventral basal, lateral basal, dorsal basal, apical, subapical and medial basal. The position and boundaries of these segments are very similar to those of the corresponding segments in the diaphragmatic lobe of the right lung. (Text-figs. 3a, 4a, b). The ventral basal, lateral basal, dorsal basal and apical segments are homologous with the anterior basal, lateral basal, posterior basal and apical segments of the left lower lobe in the human, but, as in the right lung, the subapical and medial basal segments are additional segments which are not present in the human.

To summarize: in the right lung, the apical lobe is divided into cranial and caudal segments, the cardiac lobe into lateral and medial segments, the intermediate lobe into dorsal and ventral segments and the diaphragmatic lobe into ventral basal, lateral basal, dorsal basal, apical, subapical and medial basal segments. In the left lung, the apico-cardiac lobe is divided into apical and cardiac segments, and the diaphragmatic lobe is subdivided in a similar manner to the right diaphragmatic lobe.

#### DISCUSSION

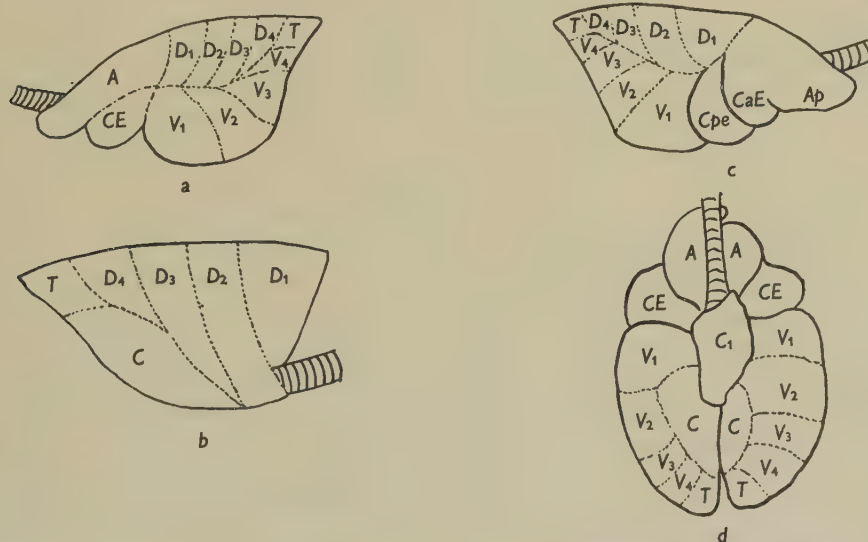
The lungs of the sheep are described in this paper as being subdivided by fissures or interlobar connective tissue planes into lobes, the right lung into four lobes named apical, cardiac, intermediate and diaphragmatic, and the left lung into two lobes named apical-cardiac and diaphragmatic. Each lobe is ventilated by a large bronchus arising either from a main bronchus, or from the trachea.

This particular subdivision of the lungs into lobes has been described by Johnston & Call (1870), Chauveau (1890), d'Hardiviller (1897) and Bressou & Vladutiu (1939), but it is not always put forth by anatomists: Sisson & Grossman (1953) mention that the right lung may be regarded as having either four or five lobes, an apical that is generally in two parts, a cardiac, a diaphragmatic and an intermediate, and that the left lung is divided into three lobes, an apical, a cardiac, and a diaphragmatic; Ellenberger & Baum (1932) are of the opinion that the right lung consists of five lobes, an apical that is in two parts, a cardiac, an intermediate and a diaphragmatic, and that the left lung consists of three lobes, an apical, a cardiac and a diaphragmatic; Paul Martin (1904) is of the opinion that the left lung only consists of two lobes when the cardiac lobe is joined to the diaphragmatic lobe.

It is clear in these latter descriptions that the lobation of the lungs has been based on the presence of fissures, without any real attempt being made to correlate these external divisions with the branching of the bronchial tree. Now, although this method may be adequate for general purposes, it is misleading and unacceptable in any consideration of the broncho-pulmonary segments, because, having regard to the definition of a lung segment, the further subdivision of the lungs into segments is dependent on the lobation. Indeed, it is for this reason that the writer introduces



the following definition of a pulmonary lobe, based on the external appearance of the lung and the divisions of the bronchial tree: a pulmonary lobe is a large area of pulmonary tissue which is ventilated by a large bronchus arising either from a main bronchus or from the trachea; it is separated from neighbouring lobes by interlobar fissures which may be continued by connective tissue planes.



Text-fig. 6. The zones of the lungs as described by Bressou & Vladutiu (1939). (a) Costal aspect, left lung. (b) Mediastinal aspect, left lung. (c) Costal aspect, right lung. (d) Diaphragmatic aspect, right and left lungs.

#### Right lung

Apical (anterior) lobe—two zones—apical (*AL*) and anterior external cardiac (*CaE*).

Cardiac (middle) lobe—posterior external cardiac (*Cpe*).

Diaphragmatic (posterior) lobe—eleven zones. Four dorsal: dorsal anterior (*D*<sub>1</sub>); dorsal antero-median (*D*<sub>2</sub>); dorsal postero-median (*D*<sub>3</sub>); dorsal posterior (*D*<sub>4</sub>). Four ventral: ventral anterior (*V*<sub>1</sub>); ventral antero-median (*V*<sub>2</sub>); ventral postero-median (*V*<sub>3</sub>); ventral posterior (*V*<sub>4</sub>). One terminal (*T*). One complementary (*C*). One internal cardiac zone (azygos lobe) *C*<sub>1</sub>.

#### Left lung

Apical (anterior lobe—two zones—apical (*A*) and external cardiac (*CE*).

Diaphragmatic (posterior) lobe—ten zones. Four dorsal (*D*<sub>1</sub>, *D*<sub>2</sub>, *D*<sub>3</sub> and *D*<sub>4</sub>) as in right lung. Four ventral (*V*<sub>1</sub>, *V*<sub>2</sub>, *V*<sub>3</sub> and *V*<sub>4</sub>) as in right lung. One terminal (*T*). One complementary (*C*).

It is on the basis of this definition that the right lung is divided into four lobes and the left lung into two lobes. The two lobes which are subject to varied descriptions are the apical lobe of the right lung and the apico-cardiac lobe of the left lung. Each is ventilated by a single bronchus, the apical lobar bronchus arising from the trachea and the apico-cardiac lobar bronchus arising from the left main bronchus, and therefore they cannot be subdivided further into lobes; the external demarcations, which in each case suggest the presence of two lobes, in fact mark the boundaries of broncho-pulmonary segments.

The lungs are then subdivided further into broncho-pulmonary segments, the right into twelve segments and the left into eight segments. It may be suggested that the lungs are composed of a greater number of segments, but in this paper the aim has been to keep the description as simple as possible, and, at the same time, to adopt a scheme and nomenclature similar to the one approved for the human lung; moreover, some degree of licence to decide on the number of segments is permissible, since, as Appleton (1945) states: 'The term broncho-pulmonary segment has been restricted arbitrarily to those relatively large portions of lung which are ventilated by bronchi that have orifices into one of the lobar bronchi.' Therefore, what actually constitutes a segment is largely a matter of opinion.

According to Bressou & Vladutiu (1939), the right lung consists of four lobes, an apical, a cardiac, an azygos (intermediate) and a diaphragmatic, and the left lung consists of two lobes, an apical and a diaphragmatic; also the lungs are subdivided into territories of ventilation or zones as shown in Text-fig. 6.

It will be noted from the figure that Bressou & Vladutiu divide the right lung into fourteen zones and the left lung into twelve zones. This scheme of division differs considerably from the one given in this paper; in the right lung, the cardiac and intermediate lobes are described as zones; in the right and left lungs, the diaphragmatic lobe is regarded as having ten zones, one of which, the complementary, is ventilated by between two and eight complementary bronchi. These bronchi are described as arising from the medial and ventral aspects of the stem bronchus, generally between the second dorsal and fourth ventral bronchi, to ventilate a large part of the mediastinal aspect of the lung. The part of the complementary zone which is ventilated by the first of the complementary bronchi is homologous with the medial basal segment as described in this paper; the remainder of the complementary zone, together with the zones ventral posterior and terminal, are included in the area called the dorsal basal segment, because it is felt that it is better to consider this area as a single segment since the bronchi which arise from the diaphragmatic lobar bronchus, after it gives off the earlier segmental bronchi, are subject to great variation.

The description of the zones in the lungs of domestic animals given by Bressou & Vladutiu is based on the scheme proposed by Lucien & Weber (1936) for the territories of ventilation in the human lung. This scheme differs considerably from the one agreed to by the Thoracic Society which, with the exception of the left upper lobe, conforms with the definition of a broncho-pulmonary segment as given by Kramer and Glass (1932); and if, as in this paper, the definition of Kramer & Glass is accepted, then an area of the pulmonary tissue which is described as a lobe cannot be described as a zone, or segment, and likewise, a zone, or segment, must be ventilated by a single bronchus.

#### SUMMARY

1. The lobation of the lungs is described; the right lung is divided into four lobes, an apical, a cardiac, an intermediate and a diaphragmatic, and the left lung is divided into two lobes, an apico-cardiac and a diaphragmatic.
2. The main branches of the bronchial tree are described with attention to the areas which they ventilate.

3. The broncho-pulmonary segments are described following a survey of the lungs of twenty sheep; the right lung is found to consist of twelve segments and the left lung of eight segments. A scheme and nomenclature similar to the international nomenclature accepted by the Thoracic Society for man is adopted.

I am most grateful to Mr T. Grahame, T.D., F.R.C.V.S., F.R.S.E., for his advice and encouragement during the preparation of this paper, and to Dr J. H. Ballantyne for allowing me facilities to complete the manuscript in the Department of Anatomy, Ontario Veterinary College. I also wish to thank Mr R. S. Hood, A.I.B.P., for his excellent photography and Mr A. Laing for his technical assistance.

#### REFERENCES

- APPLETON, A. B. (1945). The arteries and veins of the lungs. *J. Anat., Lond.*, **79**, 97-120.
- BERG, R. M., BOYDEN, E. A. & SMITH, F. R. (1949). An analysis of variations of the segmental bronchi of the left lower lobe of fifty dissected, and ten injected, lungs. *J. thorac. Surg.* **18**, 216-236.
- BOYDEN, E. A. & HARTMANN, J. F. (1946). An analysis of variations in the bronchopulmonary segments of the left upper lobes of fifty lungs. *Amer. J. Anat.* **79**, 321-360.
- BRESSOU, C. & VLADUTIU, O. (1939). La systématisation pulmonaire chez les animaux domestiques. *Bull. Acad. vet. Fr.* **12**, 203.
- BROCK, R. C. (1946). *The Anatomy of the Bronchial Tree*, 1st ed. London: Oxford University Press.
- CHAUVEAU, A. (1890). *Traité d'Anatomie Comparées des Animaux Domestiques*, 4th ed. Paris: J. and B. Baillière.
- D'HARDIVILLER, M. D. A. (1897). Développement des bronches principales chez le mouton. *C.R. Soc. Biol., Paris*, **4**, 1002, 1040 and 1054.
- ELLENBERGER, W. & BAUM, H. (1932). *Handbuch der Vergleichenden Anatomie der Haustiere*, 17th ed. Berlin: Julius Springer.
- EWART, W. (1889). *The Bronchi and Pulmonary Blood Vessels*. London: J. and A. Churchill.
- FOSTER-CARTER, A. F. (1942). The anatomy of the bronchial tree. *Brit. J. Tuberc.* **36**, 19-38.
- HUIZINGA, E. & SMELT, G. J. (1949). *Bronchography*. Assen: Van Gorcum and Co.
- JACKSON, C. L. & HUBER, J. F. (1943). Correlated applied anatomy of the bronchial tree and lungs with a system of nomenclature. *Dis. Chest*, **9**, 319-326. (Ref. Huizinga, E. & Smelt, G. J. (1949), *Bronchography*.)
- JOHNSTON, J. W. & CALL, T. J. (1870). *Descriptive Anatomy of the Horse and Domestic Animals*. Edinburgh: MacLachlan and Stewart.
- KRAMER, R. & GLASS, A. (1932). Bronchoscopic localization of lung abscess. *Ann. Otol., etc., St Louis*, **41**, 1210-1220.
- LUCIEN, M. & WEBER, P. (1936). La systématisation pulmonaire chez l'homme. Caractères généraux et morphologie de la ramescence des bronches intrapulmonaires. Leur repartition topographique. *Arch. Anat., Strasbourg*, **21**, 109-141.
- MARTIN, PAUL (1904). *Lehrbuch der Anatomie der Haustiere*, vol. II, Stuttgart: Schickhardt and Ebner.
- RAP, A. A. (1947). *Over de anatomie van de bronchiaalboom en de verdeling der longsegmenten*. Assen: Van Gorcum and Co.
- SCANNELL, J. G. (1947). A study of variations of the bronchopulmonary segments in the left upper lobe. *J. thorac. Surg.* **16**, 530-537.
- SCANNELL, J. G. & BOYDEN, E. A. (1948). A study of variations of the bronchopulmonary segments of the right upper lobe. *J. thorac. Surg.* **17**, 232-237.
- SISSON, S. & GROSSMAN, J. D. (1953). *The Anatomy of the Domesticated Animals*, 4th ed. Philadelphia: W. B. Saunders Co.
- SMITH, F. R. & BOYDEN, E. A. (1949). An analysis of variations of the segmental bronchi of the right lower lobe of fifty injected lungs. *J. thorac. Surg.* **18**, 195-215.
- THORACIC SOCIETY (1950). The nomenclature of broncho-pulmonary anatomy. *Thorax*, **5**, 222-228.



## EXPLANATION OF PLATE

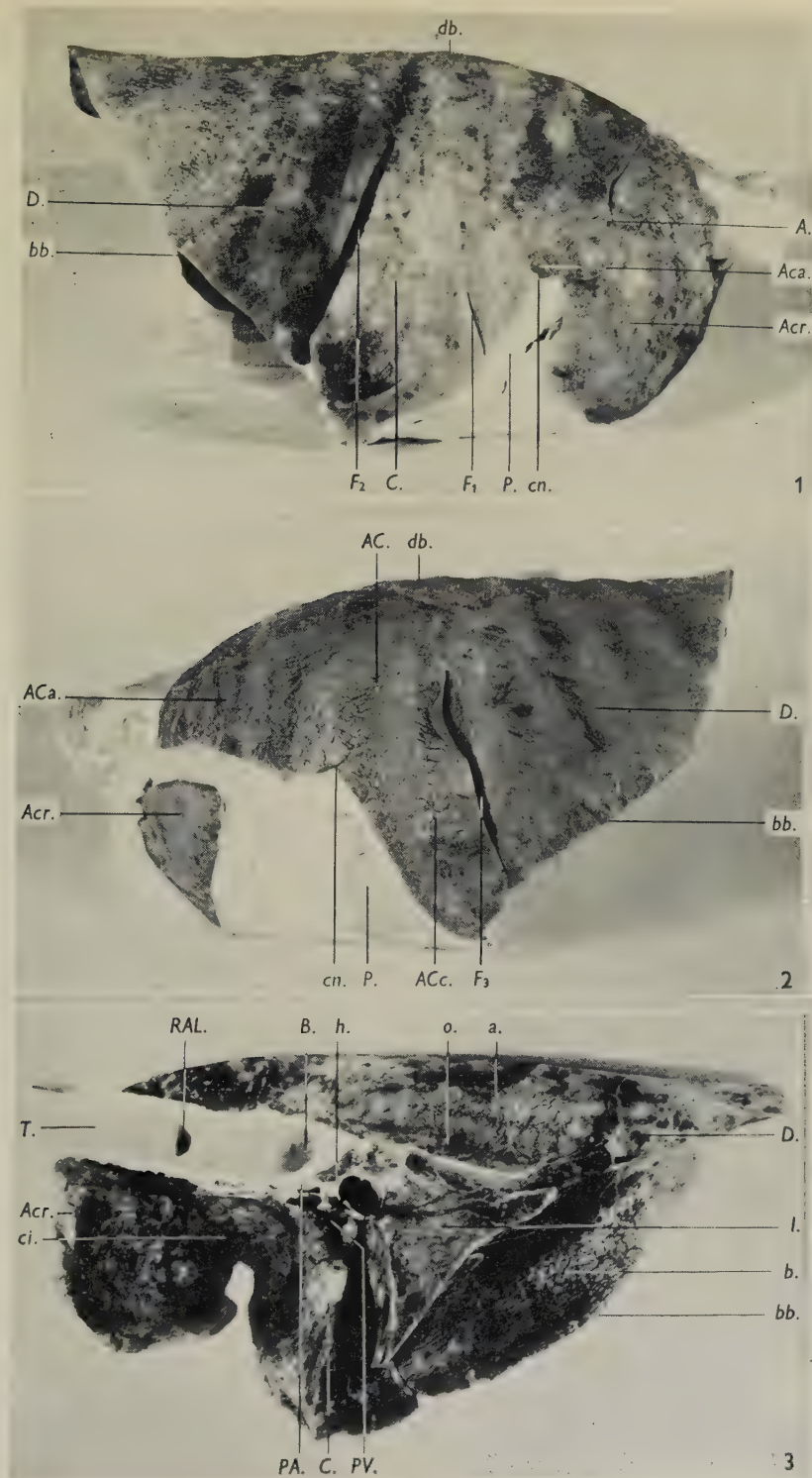
## PLATE 1

Fig. 1. Costal surface of the right lung.

Fig. 2. Costal surface of the left lung.

Fig. 3. Mediastinal surface of the right lung.

<i>A.</i>	Right apical lobe	<i>T.</i>	Trachea
<i>Acr.</i>	Cranial part of the right apical lobe	<i>a.</i>	Groove for the aorta
<i>Aca.</i>	Caudal part of the right apical lobe	<i>b.</i>	Basal surface
<i>AC.</i>	Apico-cardiac lobe	<i>bb.</i>	Basal border
<i>ACa.</i>	Apical part of the apico-cardiac lobe	<i>ci.</i>	Cardiac impression
<i>ACc.</i>	Cardiac part of the apico-cardiac lobe	<i>cn.</i>	Cardiac notch
<i>B.</i>	Right main bronchus	<i>db.</i>	Dorsal (vertebral) border
<i>C.</i>	Cardiac lobe	<i>F<sub>1</sub>.</i>	Fissure between the apical and cardiac lobes
<i>D.</i>	Diaphragmatic lobe	<i>F<sub>2</sub></i>	Fissure between the cardiac and diaphragmatic lobes
<i>I.</i>	Intermediate lobe	<i>F<sub>3</sub></i>	Fissure between the apico-cardiac and diaphragmatic lobes
<i>P.</i>	Pericardium (heart enclosed)	<i>h</i>	Hilus
<i>PA.</i>	Pulmonary artery	<i>o</i>	Groove for the oesophagus
<i>PV.</i>	Pulmonary vein		
<i>RAL.</i>	Right apical lobar bronchus		







## IN PIAM MEMORIAM

### SIR ARTHUR KEITH, F.R.S.

Sir Arthur Keith, doyen of British anatomists, died at Downe in Kent on 7 January 1955 in his 89th year. He was born on 5 February 1866 at Quarry Farm, Woodside, near Aberdeen; there and at Kinnermit, near Turiff, his boyhood was spent. Of Aberdeenshire farming stock, and the fourth son of his parents' family of ten, Arthur seemed destined to follow the family tradition. After local schooling, however, his father agreed that he should have a year at Gordon's College. Thence he proceeded to Aberdeen University wherein, after another preliminary year in the Faculty of Arts and passing the necessary examination in Greek, he began his medical studies at Marischal College in 1884.

Keith records that John Struthers, the Professor of Anatomy, was at that time 'regarded as very advanced' and 'it was he that opened the Darwinian gates for me' (*Autobiography*, p. 74). Indeed, Keith was twice prizeman in Anatomy, receiving in 1885 a copy of *The Origin of Species* and in 1886, likewise, Tylor's *Anthropology*—twin beacons that surely lighted the way to his future career. In 1888 he graduated with highest honours and the John Murray Medal as the most distinguished graduate of the year.

After a term as Demonstrator of Physiology, the 'false start' of a brief period as Assistant at the Murray Asylum in Perth, and a year's experience of general practice in Mansfield, the critical chapter of Keith's life opened with his appointment in the dual capacity of medical officer to a mining company in Siam and plant-collector assistant in the botanical survey of the Malay Peninsula. During his three years in Siam he did indeed collect some 500 plants, later used in Ridley's *Flora*; but his real interest was in the monkeys and apes, and he was soon deeply engaged in observation and dissection, amassing an abundance of notes in eight folio volumes. So, in 1891, his first scientific paper, 'Anatomical Notes on Malay Apes', was published in the relative obscurity of the *Proceedings of the Singapore Branch of the Royal Asiatic Society*. On the failure of the mining company and after various adventures and a serious illness, Keith returned to England, determined upon an anatomical career and devoting himself, at the expense of all his savings, to study for higher qualification.

There followed an austere period of industry in London, which included work under Thane at University College, intimate acquaintance with the Museum and Library of the Royal College of Surgeons, familiarity with the contents of the metropolitan medical museums and the continuation of primatological research on material from the Zoological Society's prosectorium. His second paper (the first in this *Journal*) appeared in 1894 on 'The Comparative Anatomy of the Ligaments of Man and the Ape', the dissections for which had earned for him in the previous year the first award of the Struthers Medal and Prize of the University of Aberdeen. Continuing the same line of work, he then prepared a thesis on comparative myology for which he was awarded his M.D., and in the same year he passed the final F.R.C.S. examina-

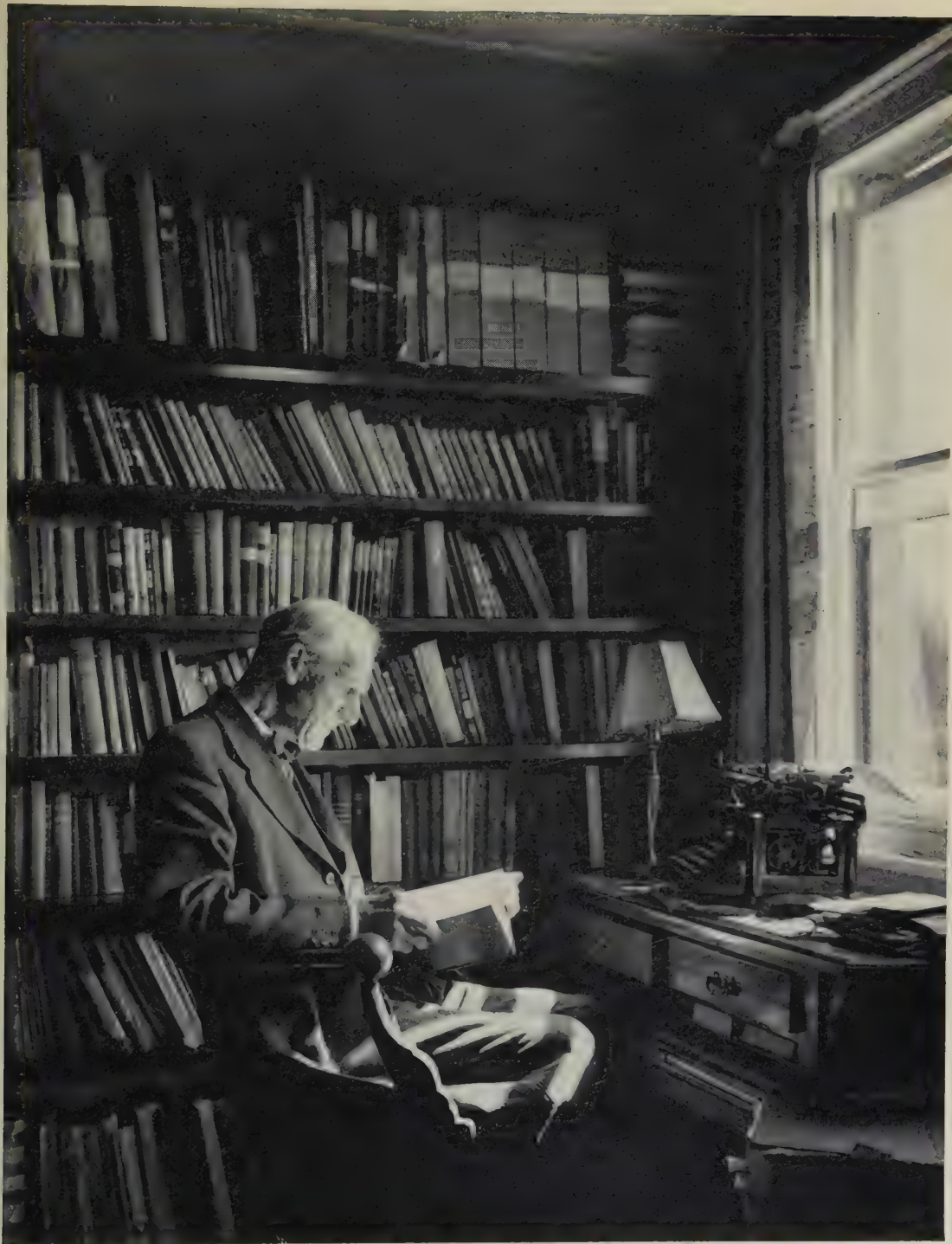
tion. His immediate objectives obtained, he now awaited opportunity to embark upon his chosen career.

In 1895 Dubois's discovery of *Pithecanthropus* prompted Keith, already a confirmed Darwinist, to begin that study of human palaeontology which was to remain his life's interest and for which he is best remembered. It occasioned also a semi-scientific article (in *Science Progress*) on the Java fossil, the beginning of a life-long, assiduously cultivated connexion with the lay press. This was the first example of that flair for the popular exposition in speech and writing of scientific matters which did much in later years to establish his national reputation.

After a short period of study under His at Leipzig (on borrowed funds) Keith secured (1895) his desired entry into the professional anatomical world by his appointment as Senior Demonstrator of Anatomy to the London Hospital Medical College at an initial salary of £75 per annum. He was then 29, in debt to friends and without teaching experience. But Aberdonian student habits stood him in good stead. He laboured diligently to prepare his classes and to perfect himself in the art of undergraduate instruction: his determination and native ability did the rest. He was soon allotted special Fellowship classes and the Curatorship of the museum, and was finally put in charge of the Department as Lecturer—extra duties and promotion which carried welcome emoluments. At that period, outside University College and King's College, anatomical teaching in the medical schools of the London hospitals, initiated by the surgeon-anatomists of the eighteenth century, was still in the hands of their successors: the well-qualified Keith proposing a career wholly devoted to Anatomy was necessarily a somewhat suspect novelty. But his strong and vivid personality, his natural charm, his knowledge and his attractive didactic style impressed his clinical confreres and won respect and admiration for the man himself and appreciation of the curricular importance of his subject. For thirteen years Keith served 'the London', stimulating successive student generations, collaborating with clinical colleagues, pursuing a variety of research projects, and securing the recognition of an ever-widening circle of professional and scientific acquaintance.

His researches at this period all had a clinical bearing: they concerned the functional anatomy of respiration, the morphology of the pelvic floor, the acquisition of the upright posture, visceral topography and the structure and development of the heart. In 1903 he had made contact with Dr (later, Sir) James Mackenzie who supplied him with pathological hearts, demanding anatomical explanation of their clinical failure, and Keith proceeded to re-investigate the atrial musculature in great detail. Then in 1905 Mackenzie consulted him about the 'bundle of His', unknown to most anatomists in this country at that time. A letter to the *Lancet* and a communication to the Anatomical Society drew attention to this hiatus in our knowledge and teaching; and Keith, having verified the existence of the His-Tawara system, returned to his examination of the atrial musculature. From this sequence of events sprang the discovery with Martin Flack in 1906 of the sinu-atrial (Keith-Flack) node—Keith's permanent memorial in anatomical history and nomenclature.

The 'London' period saw Keith's pen engaged not only on the output of scientific papers but also on the labour of book-writing. His Primary Fellowship class-notes formed the basis of his inimitable *Human Embryology and Morphology* (1901, 6th ed.



SIR ARTHUR KEITH, F.R.S.

(Facing p. 404)





1948); he edited the unsuccessful *Hughes' Manual of Practical Anatomy* and assisted Sir Frederick Treves in new editions of *Surgical Applied Anatomy*. With this remarkable industry went active participation in the business and affairs of the Anatomical Society, the Zoological Society and the Royal Anthropological Institute. When Charles Stewart's death (1908) left vacant the Conservatorship of the Hunterian Museum, Keith was the obvious choice of the Council of the Royal College of Surgeons as successor. The post—which he had long secretly coveted—was eminently congenial to Keith's interests and talents, and he served the Royal College faithfully for twenty-five years, worthily maintaining the traditions of his great predecessors in office and enhancing the reputation, range and utility of the vast collections which, during the years, had grown around the original Hunterian nucleus. With the several Museum departments ably officered by distinguished colleagues—Burne in comparative anatomy, Shattock, Beadles and Lawrence in pathology, Colyer in odontology—and supported by a skilled technical staff, Keith was now free to devote himself more fully to old interests in teratology and human morphology and to develop on a grand scale his passion for anthropology and his inquiries into problems of human ancestry. At the same time his Hunterian Lectures and Museum Demonstrations were all elaborately, even meticulously, prepared and sometimes saw publication in book form, e.g., *Menders of the Maimed* (1919), arising out of the problems posed by war injuries. In the same year his Christmas Lectures at the Royal Institution in 1916 appeared as *The Engines of the Human Body*. His Lectures and Demonstrations were mostly devoted, as had been his earlier teaching, to the practical needs of the medical student and practitioner; for as anatomist Keith never abandoned the general fellowship of Medicine and held it a cardinal duty readily and willingly to place anatomical fact or explanation at the disposal of the clinician. For this reason he chose to publish in the *Lancet* and the *British Medical Journal* papers which would have been welcomed by more purely 'scientific' periodicals.

During his benign regime important improvements were effected in the grouping and display of the Museum contents, a unique odontological department was established by agreement with the Royal Society of Medicine, the 1914–18 war years were survived without mishap, and the Army Medical War Collection, embellished by Tonks's admirable illustrations, was assembled and permanently exhibited to commemorate the military pathology of that war. Periodically specialist honorary workers were invited to devote their time and knowledge to particular Museum subsections and so to enhance the utility and attraction of these by preparing special descriptive catalogues—in reality, authoritative scientific monographs—thereon. (Thus, Alban Doran worked on the vertebrate ear and on ancient surgical instruments, T. B. Layton on the Onodi Collection, Lawford Knaggs on bone disease, V. E. Negus on comparative rhino-laryngology.) Research workers from home and overseas made repeated pilgrimage to the Museum to utilize its unique specimen-series, unrivalled in range and quality, and were accorded the fullest encouragement and facilities.

From many and very diverse sources Keith personally secured valuable Museum donations, for he possessed abundantly that essential attribute of the successful conservator—the irresistible beggar's faculty. His anthropological interests brought to the Museum human archaeological material from numerous prehistoric and his-

toric horizons, both British and foreign. Europe, Egypt, Turkestan, West and East Africa, Mesopotamia, Asia Minor and the West Indies provided their quota; from the Thames Valley gravels, from long and round barrows, from Iron and Bronze Age sites, from Saxon camps and Jutish settlements, from Romano-British and medieval excavations came a representative selection of the skeletal remains of the successive inhabitants of these islands. Probably no one, before or since, has handled so extensive an historic and geographic range of human osteology: understandably, with such material under his eye and the unique anthropological content of the Museum at his disposal, Keith rapidly gained acceptance as the arbiter upon ancient human remains and as the leading authority on the problems posed by such material—the characteristics, origin and significance of human races. In methodology he invented divers craniometrical instruments, introduced the subcerebral plane and stressed the role of ‘flumina’ and ‘pools’ of cerebrospinal fluid in the configuration, and the interpretation, of endocranial casts.

At this period of his life he made elaborately detailed studies of known human fossils and of each new one as it came to light, and he figured actively in the discussions and controversies which some of these discoveries engendered. His first book on anthropology was his *Ancient Types of Man* (1911), but in 1915 appeared his classic monograph, *The Antiquity of Man*, which established his pre-eminence in anthropology and extended his reputation beyond merely professional circles. An expanded two-volume second edition was called for in 1925, while the continued enrichment of the human palaeontological record necessitated the production of a third, and companion, volume, *New Discoveries relating to the Antiquity of Man* (1931). His minor anthropological publications cannot be detailed here, nor are they fully listed in the appended Bibliography; for these, as for much else, the catalogue of his writings, itself lacking numerous ephemeral items, but containing some 529 titles, and preserved in the Library of the Royal College of Surgeons, must be consulted.

In 1933 recurrent ill-health induced his resignation from the Conservatorship, whereupon the College Council, ever appreciative of his services and his scientific standing, appointed him Master of the Buckston Browne Research Farm at Downe, and, later, a Trustee of the Hunterian Museum. The Research Farm, munificent gift of Sir George Buckston Browne, Darwinian enthusiast and Keith’s admirer, was erected in close proximity to Darwin’s old home, Down House, itself an earlier purchase by Buckston Browne and his gift to the British Association. Declining a pressing offer to become resident custodian of Down House, Keith acquired and restored a cottage (Homefield) situate between Farm and House; and there, in close relation to the memorial to Darwin, who had been the inspiration of his life, he spent his remaining years.

Twelve months after transferring to Homefield, Lady Keith died. She was Cecilia Gray (daughter of Tom Gray, Aberdonian artist) whom Keith married in 1899. They had no children, and she devoted herself to her distinguished husband, whose career she nurtured by her self-effacing domesticity and whose *Autobiography* records her praise. She was buried in Downe churchyard. Not for another twenty-one years did her husband’s ashes join hers, years of wonderfully sustained, continuous, activity. To their very end physical vigour remained largely unimpaired and memory retained



an extraordinary range and clarity over the long life-span. Supervision of the young experimental surgeons working at the Farm, trips to London and elsewhere (in the earlier years at least), the perusal of scientific journals, visits from, and correspondence with, old friends and new, the cultivation of his small holding (including small-scale stock-raising during the 1939–45 war years), the preparation of materials for future publications, the anthropological study of the Mount Carmel fossils, the implications of new discoveries in palaeontology, the maturation and systematization of earlier evolutionary views—all this occupied an acute and well-stored mind which refused to acknowledge the insidious onset of age. When his right hand became tremulous Keith promptly learned to write left-handedly, so successfully indeed that the manoeuvre went unsuspected. His industry never slackened nor did his output diminish. Though he had already surpassed most men in the authorship of authoritative books and papers, yet in these evening years of official retirement the Master was to publish some of his finest and most memorable work.

The 1935 discovery of the Swanscombe skull stimulated him to re-survey his earlier (1914) interpretation of the Piltdown ‘fossil’, the inherent problems of which had never ceased to exercise his mind. The technique of his new assessment and the conclusions reached anent ‘*Eoanthropus*’ appeared in this *Journal* (1938, 1939). Characteristically, Keith championed his newer Piltdown reconstruction every whit as vigorously as he had his original one. To the time of the exposure of the whole Piltdown fraud, he never finally resolved his doubts about the Piltdown ‘mosaic of neanthropic and simian features’; when the fraud was exposed it took him some time to adjust himself to a distressing situation, the tragedy of which to him, he said, was the ‘loss of faith in the testimony of our fellow-workers’.

In 1939, five years’ incessant labour at Downe with Theodore D. McCown, of the University of California, culminated in a monumental joint monograph on Palestinian fossil man (*The Stone Ages of Mount Carmel. Vol. II. The Fossil Human Remains*). This represents perhaps Keith’s happiest and most enduring contribution to physical anthropology and human palaeontology, the implications of which still await their deserved recognition and appreciation.

In *Essays on Human Evolution* (1946) Keith elaborated hypotheses formulated thirty years previously and embodied his matured opinions upon racial development and sociological factors, including war, in human evolution. In 1948 came his *New Theory of Evolution*, summarizing and systematizing his personal evolutionary philosophy. The same year saw the appearance of his last anatomical work—the 6th edition of *Embryology and Morphology*, containing all the most recent bibliographical references and incorporating the latest discoveries and opinions in the embryological field. And as though this record of achievement were insufficient, there appeared in 1950 another substantial volume, his extraordinarily frank and self-revealing *Autobiography*, detailing much of the thought and activities of his long life. This he intended to be his last book: yet at his death he had written, typed and prepared for publication a still further volume, characteristically entitled *Darwin Revalued*. All this constitutes a remarkable and truly impressive performance by a man in his ninth decade, confronted also by responsibilities and restrictions during the 1939–45 war years, with the added heartache of the destruction of his beloved Museum in 1941.

Keith rendered various public services which included his membership (1913) of the Haldane Committee on London University, his advisory assistance (1917) to the Medical Board concerned with the grading of army recruits, and his membership (1924-5) of the University Grants Committee. He also served Cambridge, London and other Universities as assessor in professorial appointments. He considered himself, however, a poor and ineffectual committee man: he avoided such engagements whenever possible, for he grudged the time so consumed and taken from more congenial scientific pursuits.

He gathered numerous honours—the Fellowship of the Royal Society (1913), knighthood (1921), the Honorary Fellowship of the Royal Society of Edinburgh (1930), the Rectorship (1930-3) of his Alma Mater, together with its LL.D. and those of Birmingham and Leeds Universities, the Hon.D.Sc. of Oxford, Durham and Manchester Universities, and Honorary Membership of British and foreign learned societies—distinctions deeply appreciated and borne with characteristic modesty.

He long maintained a close connexion with the Royal Institution, not only as a frequent Lecturer therein but as its Fullerian Professor of Physiology (1917-23), Secretary (1922-6) and Treasurer (1926-9). Joining the Anatomical Society in 1893, he served later as its Secretary (1898-1901) and as its President (1918-20). For seventeen years after the *Journal of Anatomy* became the property and official organ of the Society in 1916, Keith discharged the duties of Acting Editor, and the Editorial Committee met in his room at the College. Appropriately, therefore, Vol. LXIX of the *Journal* (1934) was dedicated to him—‘A beloved Master of British Anatomy’—as ‘The Arthur Keith Volume’, in affectionate acknowledgement of his sustained and devoted service to anatomical interests.

His intimate connexion with the Royal Anthropological Institute brought him the presidency of that body (1912-14), in which capacity he delivered four presidential addresses, besides being its Huxley Memorial Lecturer in 1928. His pre-eminence in Anthropology was further recognized by his presidency of the British Association at Leeds in 1927 and by his nomination to numerous public lectureships. Thus he was the first Struthers Memorial Lecturer of the Royal College of Surgeons of Edinburgh in 1911, when he paid tribute to his original master and spoke on Anatomy in Scotland during his lifetime. Among many other similar engagements of importance he was Herter Lecturer (Johns Hopkins University, 1921), Huxley Lecturer (Birmingham University and Charing Cross Hospital, 1923), Mitchell Banks Lecturer (Liverpool University, 1923), Vicary Lecturer (Royal College of Surgeons of England, 1923, 1924), Shorstein Lecturer (London Hospital, 1924), Henderson Trust Lecturer (Edinburgh, 1924), Munro Lecturer (Edinburgh University, 1925), Lloyd Roberts Lecturer (Royal Society of Medicine, 1925) and Ludwig Mond Lecturer (Manchester University, 1928).

Some of these addresses occasioned adverse public criticism and, in certain quarters, even resentment, by reason of their outspoken opinions on the implications of his Darwinistic and ‘rationalist’ views for religion and social evolution. In his Presidential Address to the British Association in 1927 he had developed an argument from Darwinism on frankly agnostic lines. On the following Sunday, in a sermon on ‘Religion and Science’ at the official service, the preacher took him to task; and the Press resounded with that perennial topic. Next year at Manchester in his Ludwig

Mond Lecture, Keith returned to 'The Implications of Darwinism', and an unfortunate paragraph about the relation of brain and spirit was seized upon by reporters. There followed a rather unseemly series of newspaper polemics beginning with opposing articles by Sir Oliver Lodge and Sir Arthur Keith in the *New York Times*, recalling a similar controversy with Conan Doyle three years before.

Keith's views on 'War as a Factor in Racial Evolution' had been expounded in a lecture at St Thomas's Hospital as early as 1915, and in 1931, in the course of his Rectorial Address at Aberdeen, he coined the metaphorical phrase 'the pruning-hook of war', which occasioned immediate public and private reaction.

The echoes of those days soon died away, for the loss of public esteem suffered by Keith for the too vigorous promulgation of doctrines obnoxious to many was but temporary. His opinions and conclusions, even in scientific matters, were not, however, immutable. On the contrary, in all the range of his prolific writings, varying from substantial scientific contributions to the merest rationalist propaganda, it would be remarkable if many changes of opinion and even contradictions were not detectable, sometimes covered by adroit explanatory essays. His ardent enthusiasm led him also, on occasion, even in scientific matters, to make claims (as he did for the Galley Hill and Ipswich remains) that could not be justified. But on one subject—the real passion of his life—he was consistent and thoroughgoing. He had assumed the mantle of Huxley as the prophet of Darwinism, and preach he must, by tongue and by pen. It is only regrettable that without training in philosophy he allowed himself to make pronouncements on subjects to which the concepts of physical science do not apply. The easy and familiar style of Keith's writings, as exemplified in his *Autobiography*, and the rapidity of his composition, doubtless had something to do with pronouncements which more time for reflection might well have modified. An endearing foible was his constitutional incapacity for flat refusal, and his very facility in writing, as he himself acknowledged and lamented, led him to accept easily a multitude of commissions that taxed his time and energies. But, though many of his addresses and popular articles may be forgotten, his solid contributions to his subject remain. And not only anatomy and anthropology were enriched by his labours: pathology, too, owes him much, for he wrote penetratingly upon such themes as cardiac teratology, the genesis of hernia and the diseases of bone. He gave the best and most comprehensive accounts of acromegaly and progeria, and was the first to describe diaphyseal aclasis. His joint discovery of the sinu-atrial node perpetuates his memory in the history of cardiology.

Keith's achievements and distinctions did much to enhance the prestige of British anatomical and anthropological science at home and abroad, as did his influence to improve the academic standing of the professional anatomist. There can be no doubt that he has left an indelible mark on British anatomy and the study of human evolution: his best work constitutes a permanent contribution to knowledge and will form his true memorial.

In person Keith was tall and spare-framed, with a markedly aquiline profile and a characteristic mode of speech compounded curiously of alternate hesitations and bursts of forceful enunciation. The expressive mobility of his features, as of his eloquent, capable hands, escaped alike the artist's brush and the photographer's lens: none of his portraits, and but few of his photographs, capture the essentials of



his arresting personality. His most satisfactory likeness is the excellent bust executed in 1936 by Miss Kathleen Parbury, and now in the Royal College of Surgeons. Excellent, too, is the photograph\* in his study at Homefield that adorns this Notice, with its vivid impression of the octogenarian anatomist still at work. As a lecturer he captivated any audience by his innate self-dramatization and by permitting his hearers to observe, as it were, his mind at work and thus to achieve an intimate personal contact. His humour expressed itself in the rare, winsome smile and the quiet chuckle, and though never perhaps the Johnsonian 'clubbable' man, his precise and well-stocked memory rendered him a remarkable raconteur. His industry was formidable and his mind alert in appreciating the essence of a problem and in devising the speediest means of its solution. Though chronic ill-health dogged him throughout a long life, he never spared 'Brother Ass'. Grave and courteous in demeanour, of an almost Carthusian austerity, he fulfilled admirably the popular notion of the detached man of science. His superficial diffidence cloaked, however, high ambition and a keen eye to the value of publicity: he knew his own merits and expected their due recognition.

Essentially an individualist, and without the ordinary succession of students and assistants, Keith founded no 'school' in either anatomy or anthropology. Nevertheless, he exerted a continuous and not inconsiderable influence on anatomy in these islands and overseas. He was encouragingly helpful to all who consulted him, and was generous in the granting of assistance even at personal inconvenience. His most distinguished pupil was Frederic Wood Jones, of whose career, in spite of their differences of opinion on the subjects of Darwin and Lamarck, he was vastly proud. Essentially lovable, Keith evoked from his fellow anatomists an abiding affection for his person and a justifiable pride in his accomplishments.

A large congregation, representative of all his many interests (including some not usually associated with such occasions) attended the Memorial Service for Arthur Keith in St Martin-in-the-Fields on Monday, 14 February 1955—most appropriately the anniversary of the birthday of John Hunter, whose Museum he had so lovingly tended. For Arthur Keith, who also in his life and labours exhibited in high degree *praeferendum ingenium Scotorum*, was the natural disciple of John Hunter.

The Address was delivered by Keith's fellow-Aberdonian, Sir Gordon Gordon-Taylor, who permits us to quote his concluding words of appreciation:

To many like myself, however, he still remains the captivating custodian of the Museum in Lincoln's Inn Fields, the paladin of courtesy with personal magnetism; the tall lithe figure, the white coat, the handsome face, the small piping voice with the delightful Scottish accent, a momentary vision flitting like a will o' the wisp from gallery to gallery in the old College Museum before the holocaust, yet ready at all times to lend an attentive ear to some youthful enquirer for information and help and encouragement.

*Et erit in pace memoria ejus.*

J. C. BRASH

A. J. E. CAVE

\* This photograph, taken in 1949 by John Miller, appeared as a plate in the *Autobiography*. It is reproduced here by the courtesy of Messrs Watts & Co.

## SIR ARTHUR KEITH'S SELECTIVE BIBLIOGRAPHY

[Sir Arthur Keith's publications, exclusive of reviews, book prefaces and the like, exceed 500 in number and cannot be detailed here in their entirety. A complete list of them is, however, preserved for consultation in the Library of the Royal College of Surgeons of England. From this list, by courtesy of the Librarian, Mr W. R. Le Fanu, the following representative items have been selected, classified under convenient headings, and arranged thereunder chronologically. All Sir Arthur's anatomical publications are included, with but a selection of his writings on anthropological, clinical and other subjects.]

## ANATOMICAL (HUMAN AND COMPARATIVE)

1891. Anatomical notes on Malay apes. *J. R. Asiat. Soc., Straits Branch, Singapore*, 23, 77.
1894. The comparative anatomy of the ligaments of men and apes. *J. Anat. Physiol.* 28, 149.
1894. Note on the supracostalis anterior. *J. Anat. Physiol.* 28, 333.
1894. Notes on a theory to account for the various arrangements of the flexor profundus digitorum in the hand and foot of primates. *J. Anat. Physiol.* 28, 335.
1894. Supracostalis and flexor muscles. *J. Anat. Physiol.* 28 (*Proc. Anat. Soc.*), xii.
1894. The ligaments of the catarrhine monkeys, with reference to corresponding structures in man. *J. Anat. Physiol.* 28, 149.
1895. The growth of the brain in men and monkeys, with a short criticism of the usual method of stating brain-ratios. *J. Anat. Physiol.* 29, 282.
1895. The modes of origin of the carotid and subclavian arteries from the arch of the aorta in some of the higher primates. *J. Anat. Physiol.* 29, 453.
1896. A variation that occurs in the manubrium sterni of higher primates. *J. Anat. Physiol.* 30, 275.
1896. A comparison of the anomalous parts of two subjects, the one with a cervical rib, the other with a rudimentary first rib, by L. E. Hertslet, with notes on the cases by A. K. *J. Anat. Physiol.* 30, 562.
1896. Organs from dissecting-room subjects which had been preserved with formaldehyde. *J. Anat. Physiol.* 30 (*Proc. Anat. Soc.*), xi.
1896. Specimens and diagrams showing a fibrous band lying on the dorsum of the scapula superficial to the fascia covering the infraspinatus. *J. Anat. Physiol.* 30 (*Proc. Anat. Soc.*), xiv.
1896. The frequent occurrence of a divided inferior vena cava in the genus *Hylobates* (Gibbons). *J. Anat. Physiol.* 30 (*Proc. Anat. Soc.*), ii.
1896. The relative position of the spinal accessory nerve to the jugular vein and transverse process of the atlas, etc. *J. Anat. Physiol.* 30 (*Proc. Anat. Soc.*), xii.
1897. *An Introduction to the Study of the Anthropoid Apes*. London: Page and Pratt. (Previously in *Natural Science*, 1896, 9, 26, 250, 316.)
1897. The Orang-outang lately living in the Society's gardens: human and anthropoid types of hair-arrangement on the limbs. *Proc. Zool. Soc. Lond.* 1, 721.
1898. Abdominal and thoracic viscera of fourteen bodies hardened *in situ* by injecting the bodies with a solution containing formalin. *J. Anat. Physiol.* 32 (*Proc. Anat. Soc.*), iii.
1898. Abdominal and thoracic viscera of an Orang. *J. Anat. Physiol.* 32 (*Proc. Anat. Soc.*), iii.
1898. A preliminary investigation of the influence of body-posture on the position and shape of abdominal and thoracic organs. *J. Anat. Physiol.* 32, 451.
1899. On the chimpanzees and their relationship to the gorilla. *Proc. Zool. Soc. Lond.* 1, 296.
1899. Position and manner of fixation of the liver of primates, and on the part these factors played in the lobulation of the liver. *J. Anat. Physiol.* 33 (*Proc. Anat. Soc.*), xi.
1900. Specimen of the uterus of a *Macacus rhesus*, nearly at the full term of pregnancy. *J. Anat. Physiol.* 34 (*Proc. Anat. Soc.*), xlv.
1900. The anatomy and nature of two acardiac acephalic fetuses. *Trans. obstet. Soc. Lond.* 42, 99.
1901. Sir Frederick Treves's *Surgical Applied Anatomy*, 4th ed., revised with the assistance of A. K.; 5th ed. by A. K. 1907; 6th ed. by A. K. 1916; 7th ed. by A. K. and W. C. Mackenzie 1918.

1901. Specimen of a double kidney. *J. Anat. Physiol.* **35** (*Proc. Anat. Soc.*), xi.
1901. On the relations of the gall-bladder and the bile ducts. *J. Anat. Physiol.* **35** (*Proc. Anat. Soc.*), xii.
1902. (With F. Wood Jones.) A note on the fundus of the human stomach. *J. Anat. Physiol.* **36** (*Proc. Anat. Soc.*), xxxiv.
1902. *A Manual of Practical Anatomy*, parts 1-3. By A. W. Hughes and A. Keith. London: Churchill.
1902. The significance of certain features and types of the external ear. *Nature, Lond.*, **65**, 16.
1902. Inflation of the nasal canal in the skulls of adult gorillas and chimpanzees, and the relative development of the sinus maxillaris and inferior meatus in man and apes. *J. Anat. Physiol.* **36** (*Proc. Anat. Soc.*), xlvii.
1902. *Human Embryology and Morphology*. 2nd ed. 1904; 3rd ed. 1913; 4th ed. 1921; 5th ed. 1933; 6th ed. 1948. London: Arnold.
1902. The relationship of the eruption of the permanent molar teeth to the expansion of the maxillary sinus (antrum of Highmore). *Brit. J. dent. Sci.* **45**, 529.
1902. On the relationship of man to the higher primates. (Hunterian Lectures.) *J. Anat. Physiol.* **36**, 93.
1903. Contributions to the human mechanism of respiration. *J. Anat. Physiol.* **37** (*Proc. Anat. Soc.*), li.
1903. The anatomy of the valvular mechanism round the venous orifices of the right and left auricles, with some observations on the morphology of the heart. *J. Anat. Physiol.* **37** (*Proc. Anat. Soc.*), ii.
1903. The extent to which the posterior segments of the body have been transmuted and suppressed in the evolution of man and allied primates. *J. Anat. Physiol.* **37**, 18 (*Proc. Anat. Soc.*), lxxv.
1903. Anatomical evidence as to the nature of the caecum and appendix. *Lond. Hosp. Gaz.* **10**, 62; *J. Anat. Physiol.* **38** (*Proc. Anat. Soc.*), vii.
1904. (With A. Shillitoe.) The preputial or odoriferous glands of man. *Lancet*, **1**, 146.
1904. Muscular structures of the heart. (Abstract of Hunterian Lectures.) *Lancet*, **1**, 555, 629, 703.
1905. Demonstration of the development and morphology of the diaphragm. *J. Anat. Physiol.* **39** (*Proc. Anat. Soc.*), ii.
1905. Exhibition of thirty malformed human hearts. *J. Anat. Physiol.* **39** (*Proc. Anat. Soc.*), xiv.
1905. The nature of the mammalian diaphragm and pleural cavities. *J. Anat. Physiol.* **39**, 243.
1906. Malformations of the bulbus cordis: an unrecognized division of the human heart. In *Studies in Pathology: Quater-Centenary Publications, University of Aberdeen*, 55.
1906. The relative brain weights of man and woman. *Brit. med. J.* **1**, 291, 474.
1906. The auriculo-ventricular bundle of His. *Lancet*, **1**, 623.
1906. (With M. W. Flack.) The auriculo-ventricular bundle of the human heart. *Lancet*, **2**, 359.
1907. (With J. E. Spicer.) Three cases of malformation of the tracheo-oesophageal septum. *J. Anat. Physiol.* **41**, 52.
1907. (With M. W. Flack.) The form and nature of the muscular connections between the primary divisions of the vertebrate heart. *J. Anat. Physiol.* **41**, 172.
1908. An account of the structures concerned in the production of the jugular pulse. *J. Anat. Physiol.* **42**, 1.
1908. A method of indicating the position of the diaphragm and estimating the degree of visceroptosis. *J. Anat. Physiol.* **42**, 26.
1908. Malformations of the hind end of the body. *Brit. med. J.* **2**, 1736, 1804, 1857.
1909. Malformations of the heart. (Hunterian Lectures.) *Lancet*, **2**, 359, 433, 519.
1909. The mechanism of respiration in man. In L. Hill, *Further Advances in Physiology*, p. 182. London: Arnold.
1909. Congenital malformations of palate, face and neck. *Brit. J. dent. Sci.* **52**, 865, 913; and *Brit. med. J.* **2**, 310, 346, 438.
1910. Abnormal ossification of Meckel's cartilage. *J. Anat. Physiol.* **44**, 151.
1910. Constrictions and occlusions of the alimentary tract of congenital or obscure origin. *Brit. med. J.* **1**, 301.
1910. (With I. MacKenzie.) Recent researches on the anatomy of the heart. *Lancet*, **1**, 101.
1910. Diverticula of the alimentary tract of congenital or of obscure origin. *Brit. med. J.* **1**, 376.
1911. An inquiry into the nature of skeletal changes in acromegaly. *Lancet*, **1**, 993.
1912. Six specimens of abnormal heart. *J. Anat. Physiol.* **46**, 211.



1912. The functional nature of the caecum and appendix. *Brit. med. J.* 2, 1599.
1912. *The Human Body*. London: Williams and Norgate. (Home University Library no. 57.)
1913. Certain factors in tooth eruption. *Dent. Rec.* 33, 769.
1913. Abnormal crania—achondroplastic and acrocephalic. *J. Anat. Physiol.* 47, 189.
1914. The evolution, anatomy and diseases of the anthropoid apes. (Abstracts of Hunterian Lectures.) *Med. Pr.* 97, 222.
1914. The influence of internal secretions in regulating the growth of the jaws. *Int. Dent. Cong.* 6, London.
1915. A preliminary account of a search for a representation of the nodal system of the heart in the muscular walls of the alimentary tract. *J. Anat., Lond.*, 50 (*Proc. Anat. Soc.*), 1.
1915. A method of preparing dry specimens. *J. Anat., Lond.*, 50 (*Proc. Anat. Soc.*), 17.
1917. Lectures on the anatomical and physiological principles underlying the treatment of injuries to muscles, bones and joints. *Brit. med. J.* 2, 711, 785, 824, 858; 1918, 1, 14; *Med. Pr.* 104, 412, 468, 488; 1918, 105, 42.
1918. The functional anatomy of the heart. (Harveian Lecture.) *Brit. med. J.* 1, 361.
1918. (With Sir T. Wrightson.) Demonstration of a new theory of hearing. *Proc. R. Soc. Med.* 12 (Otolaryng. Sect.), 80.
1918. On the structures concerned in the mechanism of hearing: appendix, pp. 161–254, with prefatory letter pp. 156–160, in Sir T. Wrightson, *An Enquiry into the Analytical Mechanism of the Internal Ear*.
1918. Bone growth and bone repair. *Brit. J. Surg.* 5, 685; 6, 19, 160.
1919. *Menders of the Maimed, the Anatomical and Physiological Principles underlying the Treatment of Injuries to Muscles, Nerves, Bones and Joints*. London: Frowde. Reprinted—Philadelphia: J. B. Lippincott, 1952.
1919. *The Engines of the Human Body*. (Royal Institution Christmas Lectures 1916–17.) London: Williams and Norgate. 2nd ed. 1925. Swedish translation 1934.
1921. The disappearance and reappearance of the human tail. *Nature, Lond.*, 106, 845; 107, 487.
1922. (With G. G. Champion.) A contribution to the mechanism of growth of the human face. *Dent. Rec.* 42, 61; *Int. J. Orthod.* 8, 607.
1923. Man's posture: its evolution and disorders. *Brit. med. J.* 1, 451, 499, 545, 587, 624, 669.
1924. Concerning certain structural changes which are taking place in our jaws and teeth. *Brit. J. dent. Sci.* 45, 1243.
1924. The fate of the bulbus cordis in the human heart. (Schorstein Lecture.) *Lancet*, 2, 1267.
1925. Bone-formation in the eruption of teeth. *J. Anat., Lond.*, 59 (*Proc. Anat. Soc.*), 72.
1925. Bone preparations from pigs fed on madder. *J. Anat., Lond.*, 59 (*Proc. Anat. Soc.*), 97.
1925. Evolution of binocular vision. *Lancet*, 2, 201.
1925. The nature of man's structural imperfections. (Lloyd Roberts Lecture, Royal Society of Medicine.) *Brit. med. J.* 2, 929; *Lancet*, 2, 1047; *Nature, Lond.*, 116, 821, 867; *Canad. med. Ass. J.* 16, 19.
1926. Gorilla and man as contrasted forms. *Lancet*, 1, 490.
1926. The place of anatomy in medicine. *Brit. med. J.* 2, 409.
1927. Concerning the origin and nature of osteoblasts. *Proc. R. Soc. Med.* 21, (Surgery Sect.), 1.
1929. The history of the human foot and its bearing on orthopaedic practice. (Third H.O. Thomas Lecture, Liverpool Medical Institution.) *J. Bone Jt. Surg.* 11, 10.
1932. Malformations of the human body from a new point of view. (Hunterian Lectures.) *Brit. med. J.* 1, 387, 435, 489.
1941. Concerning the origin of certain malformations of the face, head and foot. *Brit. J. Surg.* 28, 173.
1949. An account of five Huxleyan plates illustrating the craniology of young anthropoid apes. *Proc. Zool. Soc. Lond.* 119, 839.

## ANTHROPOLOGICAL

1895. *Pithecanthropus erectus*—a brief review of human fossil remains. *Science Progress*, 3, 348.
1906. The result of an anthropological investigation of the external ear. *Proc. Anat. Anthropol. Soc., Univ. Aberdeen*, no. 217.
1906. Were the ancient Egyptians a dual race? *Man*, 6, 3.
1910. Buffon's engraving of a piebald negress. *Brit. med. J.* 2, 658.

1910. Description of a new craniometer and of certain age-changes in the anthropoid skull. *J. Anat. Physiol.* **44**, 251.
1910. The negro and negroid races. *Med. Pr.* **91**, 218.
1910. The position of the Negro and Pygmy amongst human races. (Abstract of Hunterian Lectures.) *Nature, Lond.*, **84**, 54.
1910. A new theory of the descent of man. *Nature, Lond.*, **85**, 206, 509.
1911. The anthropology of the ancient British races. (Abstract of Hunterian Lectures.) *Lancet*, **1**, 722.
1911. *Ancient Types of Man*. London and New York: Harper.
1911. Discovery of the teeth of palaeolithic man in Jersey. *Nature, Lond.*, **86**, 414.
1911. (With F. H. S. Knowles.) Description of teeth of palaeolithic man from Jersey. *J. Anat. Physiol.* **46**, 12; *Bull. Soc. jersiaise*, **37**.
1911. Cranium of the Cro-Magnon type found by Mr W. M. Newton in a gravel terrace near Dartford. *Rep. Brit. Ass.* p. 516.
1911. On certain physical characters of the negroes of the Congo Free State and Nigeria; being a report on material supplied by E. Torday, T. A. Joyce, P. A. Talbot and Frank Corner. *J. R. anthrop. Inst.* **41**, 40.
1911. The problem of *Pithecanthropus*. *Nature, Lond.*, **87**, 49.
1912. Modern problems relating to the antiquity of man. *Rep. Brit. Ass.* p. 753; *Brit. med. J.* **2**, 669; *Lancet*, **2**, 807.
1912. Certain phases in the evolution of man. (Hunterian Lectures.) *Brit. med. J.* **1**, 734, 788; *Med. Pr.* **93**, 244, 271; Abstract, *Lancet*, **1**, 775.
1912. (With J. Reid Moir.) An account of the discovery and characters of a human skeleton found beneath a stratum of chalky boulder clay near Ipswich. *J. R. anthrop. Inst.* **42**, 345; *Proc. Prehist. Soc. E. Anglia*.
1913. Problems relating to the teeth of the earlier forms of prehistoric man. *Proc. R. Soc. Med.* **6** (Odontological Sect.), 103.
1913. Moriori in New Zealand. *Man*, **13**, 171.
1913. The present problems relating to the origin of modern races. *Lancet*, **2**, 1050; and as a pamphlet. London: John Bale, Sons and Danielsson. 16 pp.
1914. The Piltdown skull and brain cast. *Nature, Lond.*, **92**, 197, 292, 345.
1914. The reconstruction of fossil human skulls. (Presidential address, Royal Anthropological Institute.) *J. R. anthrop. Inst.* **44**, 12.
1915. The bronze-age invaders of Britain. (Presidential address, Royal Anthropological Institute.) *J. R. anthrop. Inst.* **45**, 12; *Nature, Lond.*, **94**, 657.
1915. *The Antiquity of Man*. London: Williams and Norgate. 519 pp. New (second) edition 1925, 2 vols. (Reissued 1929.)
1915. War as a factor in racial evolution. *St Thom. Hosp. Gaz.* **25**, 153.
1916. Lo schema dell'origine umana. (Volume giubilare in onore di Giuseppe Sergi.) *Riv. antrop. Roma*, **20**, 3.
1916. On certain factors concerned in the evolution of the human races. (Presidential address, Royal Anthropological Institute.) *J. R. anthrop. Inst.* **46**, 10.
1917. The physical characteristics of two Pitcairn Islanders. *Man*, **17**, 121.
1918. The ethnology of Scotland. *Nature, Lond.*, **100**, 85.
1918. Discovery of Neanderthal man in Malta. *Nature, Lond.*, **101**, 404.
1919. The differentiation of mankind into racial types. (Presidential address, Section of Anthropology, British Association.) *Rep. Brit. Ass.* p. 275; *Lancet*, **2**, 553; *Nature, Lond.*, **104**, 301; *Rev. gén. Sci. pur. appl.* **20**, 610.
1919. Nationality and race from an anthropologist's point of view. (Robert Boyle Lecture, Oxford University Junior Scientific Club.) Oxford: Milford.
1921. The discovery of fossil remains of man in Java, Australia and South Africa. *Nature, Lond.*, **106**, 603.
1921. Darwin's theory of man's origin in the light of present day evidence. *Med. Pr.* **111**, 251, 271, 315, 335.
1921. The origin of the Scottish people. *Rep. Brit. Ass.* p. 439; *Nineteenth Century and After*, **91**, 819; *Nature, Lond.*, **108**, 548.
1922. The evolution of human races in the light of the hormone theory; racial status and form of body. *Bull. Johns Hopk. Hosp.* **33**, 155, 195.

1922. The stature of the Scottish people. *Nature, Lond.*, **110**, 8.
1923. The adaptational machinery concerned in the evolution of man's body. (Huxley Lecture, Charing Cross Hospital Medical School.) *Nature, Lond.*, **112**, 257.
1924. Neanderthal man in Malta. *J. R. anthrop. Inst.* **54**, 251.
1925. The fossil anthropoid ape from Taungs. *Nature, Lond.*, **115**, 234; **116**, 11, 462.
1925. The new missing link. *Brit. med. J.* **1**, 325.
1925. The Galilean skull. *Nature, Lond.*, **116**, 286.
1925. Concerning the rate of man's evolution. *Proc. Roy. Inst.* **24**, 571; *Nature, Lond.* **116**, 317.
1927. The drift of modern anthropology. *Brit. med. J.* **1**, 149.
1927. A report on the Galilee skull. In F. Turville-Petre *Researches in Pre-historic Galilee* (British School of Archaeology in Jerusalem.) London: Council of the School.
1927. Reports on the human remains. In H. R. Hall and C. L. Woolley, *Ur excavations*, **1**, 214; **1934**, **2**, 400. Oxford, for the British Museum.
1927. Darwin's theory of man's descent as it stands today. (Presidential address, British Association, Leeds.) *Rep. Brit. Ass.* **95**, 1; *Brit. med. J.* **2**, 439; *Lancet*, **2**, 485; *Nature, Lond.*, **120**, Supplement; *Science*, **66**, 201.
1927. Concerning man's origin. (Presidential address, British Association.) London: Watts; New York: Putnam; Spanish translation by E. P. Reed, Valparaiso, 1930.
1928. The evolution of the human races. (Huxley Memorial Lecture, Royal Anthropological Institute.) *J. R. anthrop. Inst.* **58**, 305.
1929. Human skulls from ancient cemeteries in the Tarim basin. *J. R. anthrop. Inst.* **59**, 149.
1929. Fossil man of Peking. *Lancet*, **2**, 683.
1930. Anthropology old and new. (Beddoe Memorial Lecture, British Association.) *Bristol med.-chir. J.* **47**, 287; German translation, *Anthrop. Anz.* **7**, 198.
1930. Recent discoveries of fossil man. *Nature, Lond.*, **125**, 935.
1931. *Ethnos, or the Problem of Race Considered from a New Point of View*. London: Kegan Paul.
1931. *New Discoveries Relating to the Antiquity of Man*. London: Williams and Norgate.
1931. The evidence of palaeontology with regard to evolution. *Rep. Brit. Ass.* p. 47.
1931. Fossil remains of early man. *Rep. Brit. Ass.* p. 361.
1931. The evolution of human races, past and present. *Early Man, his Origin, Development and Culture*, by G. Elliot Smith and others. London: Benn.
1932. Neanderthal man in Palestine. *Nature, Lond.*, **129**, 712.
1932. The late palaeolithic inhabitants of Palestine. *Nature, Lond.*, **130**, 284.
1932. The Aryan theory as it stands today. (Eleventh Frazer Lecture, Glasgow.) *The Frazer Lectures 1922-32*, edited by W. R. Dawson. London: Macmillan.
1932. (With W. M. Krogman.) The racial characters of the southern Arabs. In Bertram Thomas, *Arabia Felix*. London: Jonathan Cape.
1934. *The Construction of Man's Family Tree*. London: Watts.
1935. Conceptions of man's ancestry. *Nature, Lond.*, **135**, 705.
1936. History from caves: a new theory of the origin of the modern races of mankind. (Presidential address, Speleological Association.) *Nature, Lond.*, **138**, 194; *Caves and Caving*, **1**, 1; and as a pamphlet.
1938. The prehistoric people of Palestine. *Brit. med. J.* **1**, 390; *Nature, Lond.*, **141**, 340.
1938. The Florisbad skull. *Nature, Lond.*, **141**, 1010.
1939. A resurvey of the anatomical features of the Piltdown skull, with some observations on the recently discovered Swanscombe skull. *J. Anat., Lond.*, **73**, 155, 234.
1939. (With T. D. McCown.) *The Stone Ages of Mount Carmel*, Vol. 2: *The Fossil Human Remains from the Levallois-Mousterian*. Oxford: Clarendon Press.
1940. The men of Lachish. *Palestine Exploration Fund Quart.* **72**, 7.
1940. Fifty years ago. *Amer. J. phys. Anthropol.* **26**, 251.
1944. Evolution of modern man (*Homo sapiens*). *Nature, Lond.*, **153**, 742.
1945. Antiquity of man in Australia. *Man*, **45**, 116.
1946. *Essays on Human Evolution*. London: Watts.
1947. Australopithecinae or Dartians. *Nature, Lond.*, **159**, 377.
1948. *A New Theory of Human Evolution*. London: Watts.
1951. The dentition of Australopithecinae. *Man*, **51**, 70.
1952. Some anthropological notes on Darwin and on members of his family. *Man*, **52**, 181.



## CLINICAL AND PATHOLOGICAL

1899. (With H. M. Rigby.) Comparative study of the destructive effects of modern military bullets. *Lancet*, 2, 1499.
1902. The anatomy of Glenard's disease. *Lond. Hosp. Gaz.* 9, 55.
1903. On the nature and anatomy of enteroptosis (Glenard's disease). (Hunterian Lectures.) *Lancet*, 1, 631, 709.
1904. (With C. W. Mansell-Moullin.) Notes on a case of backward dislocation of head of humerus caused by muscular action. *Lancet*, 1, 496.
1904. The painful pronation of childhood. *Lancet*, 1, 1015.
1904. Syphonage in the great intestine. *Lancet*, 1, 1081, 1235.
1906. (With R. M. Goring.) A case of torsion of the testicle. *Lancet*, 1, 370.
1906. The 'saccular' theory of hernia. *Lancet*, 2, 1398.
1907. Partial deficiency of the pericardium. *J. Anat. Physiol.* 41, 6.
1907. (With J. E. Spicer.) Three cases of malformation of the tracheo-oesophageal septum. *J. Anat. Physiol.* 211, 52.
1907. Visceroptosis. In C. Allbutt and H. D. Rolleston, *System of Medicine*, 3, 860; Anatomy of the liver, *ibid.* 1908, 4 (i), 3; Hepatoptosis, *ibid.* 11.
1908. The results of some enquiries into the Schäffer method of performing respiration on the apparently drowned. *Lond. Hosp. Gaz.* 14, 206.
1909. Discussion on the nature of visceroptosis. *Lond. Hosp. Gaz.* 16, 1.
1909. The mechanism underlying the various modes of artificial respiration practised since the foundation of the Royal Humane Society in 1774. (Hunterian Lectures.) *Lancet*, 1, 745, 825, 895.
1910. Constrictions and occlusions of the alimentary tract of congenital or obscure origin. *Brit. med. J.* 1, 301.
1910. Diverticula of the alimentary tract of congenital or obscure origin. *Brit. med. J.* 1, 376.
1910. (With Alban Doran.) Specimens illustrating cysts of the female appendages. *J. Obstet. Gynaec., Brit. Emp.*, 18, 246.
1910. Remarks on diaphragmatic hernia. *Brit. med. J.* 2, 1297.
1911. (With A. R. Tweedie.) Ectopia of the pituitary, with other congenital anomalies of the nose, palate and upper lip. *Proc. R. Soc. Med.* 4, (Laryngology Sect.), 47.
1911. The nature and cause of the common forms of hernia. *Med. Pr.*, 92, 495.
1912. Drowning, in C. C. Choyce, *System of Surgery*, 1, 606. London: Cassell.
1913. Progeria and ateleiosis. *Lancet*, 1, 305.
1914. The nature of peritoneal adhesions. *Lancet*, 2, 362.
1915. An account of six specimens of great bowel removed by operation, etc. *Brit. J. Surg.* 2, 576.
1915. A new theory of the causation of enterostasis. (Cavendish Lecture.) *Lancet*, 2, 371; *Med. Pr.* 100, 72; *West Lond. Med. J.* 20, 149.
1917. Loose bullets and foreign bodies in the heart. *Brit. med. J.* 1, 278.
1918. The introduction of the modern practice of bone-grafting. *Lancet*, 1, 210.
1918. The orthopaedic practice and principles of certain American surgeons. *Med. Pr.* 105, 141.
1918. Movement as a means of treatment. *Med. Pr.* 105, 159.
1918. Wolff's law of bone transformation. *Lancet*, 1, 250.
1919. (With M. E. Hall.) Bones showing the effects of gunshot injuries in the Army Medical Collection. *Brit. J. Surg.* 6, 537.
1919. The true nature of multiple exostoses. *Med. Pr.* 108, 485; *Trans. Med. Soc. Lond.* 43, 67.
1920. (With M. E. Hall.) Specimens of gunshot injuries of the face and spine. *Brit. J. Surg.* 7, 55.
1920. (With M. E. Hall.) Specimens of gunshot injuries of the long bones, to show the type of fracture produced. *Brit. J. Surg.* 7, 149.
1920. (With M. E. Hall.) Specimens of long bones showing the processes of infection and repair. *Brit. J. Surg.* 7, 302.
1921. (With J. J. MacDonnell.) Case of transposition of viscera showing an essentially bicameral heart. *Proc. R. Soc. Med.* 14 (Medicine Sect.), 1.
1921. (With W. G. Spencer.) Intestinal stasis followed by cystic dilatation of the caecum with intestinal obstruction, etc. *Brit. J. Surg.* 8, 452.
1921. Evolutionary wounds. *Brit. med. J.* 2, 137.

1924. On the origin and nature of hernia. (Mitchell Banks Lecture, Liverpool University.) *Brit. J. Surg.* **11**, 455.
1926. (With B. Myers.) Case of congenital cyanosis. *Proc. R. Soc. Med.* **19** (Clinical Sect.), 43.
1950. Experimental production of malignant tumours of bone. *Ann. Roy. Coll. Surg. Engl.* **7**, 34.
1950. An enquiry into the causation of peptic ulcers. *Ann. R. Coll. Surg. Engl.* **7**, 163.
1953. Visceroptosis. *Lancet*, **2**, 450.

## HISTORICAL

1908. Human anatomy in England during the nineteenth century. *Lancet*, **1**, 1.
1911. The position of Sir Charles Bell amongst anatomists. *Lancet*, **1**, 290.
1911. Charles Bell and the motor and sensory functions of spinal nerves. *Lancet*, **1**, 543, 764, 901.
1912. Anatomy in Scotland during the lifetime of Sir John Struthers (1823–1889). (First John Struthers Lecture.) *Edinb. med. J.* **8**, 7.
1912. The Bell-Magendie controversy. *Lancet*, **2**, 968.
1913. History and nature of the Napoleonic specimens in the Museum of the Royal College of Surgeons of England. *Brit. med. J.* **1**, 53; *Lancet*, **1**, 187.
1913. The post-mortem examination of Napoleon. *Brit. med. J.* **1**, 259.
1916. Sir William Turner as anatomist and anthropologist. *Brit. med. J.* **1**, 327 (and 1920, **1**, 439.)
1918. Bone-setting, ancient and modern. *Med. Pr.* **106**, 362, 382, 399, 482.
1918. Edinburgh University and anatomical nomenclature. *Brit. med. J.* **2**, 699.
1919. John Hunter's beard and mask. *Lancet*, **2**, 710.
1919. The cradle of the Hunterian school. In *Contributions to Medical and Biological Research dedicated to Sir William Osler*, **1**, 88. New York: Hoeber.
1919. What did John Hunter do for medicine? *Brit. med. J.* **2**, 485.
1921. Galton's place among anthropologists. *Eugen. Rev.* **12**, 14.
1921. The principles and practice of Hugh Owen Thomas. In Sir Robert Jones's *Orthopaedic Surgery of Injuries*, **1**, 3. London: Oxf. Univ. Press.
1923. The life and times of William Clift, first Conservator of the Museum of the Royal College of Surgeons of England. (Thomas Vicary Lecture.) *Brit. med. J.* **2**, 1127; *Lancet*, **2**, 1329.
1924. Richard Owen as Conservator of the Royal College of Surgeons Museum. (Thomas Vicary Lecture.) *Brit. med. J.* **2**, 890.
1924. Phrenological studies of the skull and endocranial casts of Sir Thomas Browne. (Henderson Trust Lecture, Edinburgh University.) Edinburgh: Oliver and Boyd.
1925. The skull and ancestry of Robert the Bruce. *Nature, Lond.*, **115**, 303, 572.
1925. Huxley as an anthropologist. *Nature, Lond.*, **115**, 719.
1925. Huxley's racial lineage. *Nineteenth Century and After*, **97**, 862.
1927. The brain of Anatole France. *Brit. med. J.* **2**, 1048.
1928. A discourse on the portraits and personality of John Hunter. *Brit. med. J.* **1**, 205.
1928. Bi-centenary of John Hunter. *Nature, Lond.*, **121**, 210.
1928. The Hunters and the Hamiltons: some unpublished letters. *Lancet*, **1**, 354.
1928. An appreciation of Harvey as anatomist. *Lancet*, **1**, 999.
1928. Progress of anatomy. *J. Anat., Lond.*, **62**, 241.
1930. The genius of William Bowman. (Bowman Lecture, Ophthalmological Society.) *Trans. Ophthalm. Soc.* **50**, 32; *Brit. med. J.* **1**, 701.
1931. Fresh light on John Abernethy. *St Bartholomew's Hosp. J.* **38**, 151.
1934. John Hunter. *Med. Pr.* **188**, 498. (Reprinted in *British Masters of Medicine*, 1936. London: Baillière.)
1950. *An Autobiography*. London: Watts.

## VARIA

- 1897–8. (With F. G. Parsons.) Annual reports of the committee of collective investigation of the Anatomical Society. Nos. 6–7, 1895–6, 1896–7. *J. Anat. Physiol.* **31**, 31; 1898, **32**, 164.
1902. The recruiting sergeants of Esculapius. *Lond. Hosp. Gaz.* **8**, 177.
1910. *Illustrated Guide to the Museum of the Royal College of Surgeons of England*. London: Taylor and Francis.
1913. Medical Museum of the International Medical Congress. *Lancet*, **2**, 101.

1914. An anthropological study of some portraits of Shakespeare and of Burns. *Proc. Roy. Inst.* **21**, 23; *Brit. med. J.* **1**, 461.
1915. Soldiers as anthropologists. *Nature, Lond.*, **94**, 391.
1917. The Basle anatomical nomenclature. *Brit. med. J.* **2**, 134.
1919. The perception of sound. *Nature, Lond.*, **102**, 164.
1920. The art of hearing. *Lancet*, **2**, 330.
1923. Note on skiagraphs of thorax of men living at high altitudes. (Appendix IV to Sir Joseph Barcroft's report on the effects of high altitude.) *Phil. Trans. B*, **211**, 472.
1923. Teleology and evolution. *Lancet*, **2**, 50.
1924. The ideal tooth (Royal Dental Hospital address.) *Lancet*, **2**, 740.
1925. Evolution and intellectual freedom. *Nature, Lond.*, **116**, 75.
1926. The place of anatomy in medicine. *Brit. med. J.* **2**, 409.
1930. Inexorability of law of evolution as manifest in modern medicine. (Medical Society of London Oration.) *Trans. Med. Soc. Lond.* **55**, 279; *Lancet*, **1**, 1053; *Brit. med. J.* **1**, 893.
1942. A post-script to Darwin's 'Formation of mould through the action of earthworms'. *Nature, Lond.*, **149**, 716.
1951. A Hunterian specimen and its problem. *Ann. Roy. Coll. Surg. Engl.* **8**, 166.
1955. *Darwin Revalued*. London: Watts. (In press at Sir Arthur's death.)



## IN MEMORIAM

JAMES WHILLIS, M.D., M.S., F.R.C.S.

Professor James Whillis's untimely death at the age of 54 came as an unexpected shock to his colleagues and friends, and brought with it a deep sense of personal bereavement. Born in Newcastle upon Tyne in September, 1900, he was educated at Carlisle Grammar School and the University of Durham, where he graduated M.B., B.S., in 1922. The distinction which had marked his career as a student was capped by the award of the Philipson Scholarship in Medicine and Surgery, and this clearly marked him out as the best man of his year. At that particular time there was a shortage of house-officers, and in his final year as a student he acted as unqualified house surgeon to Sir Joseph Leech. But, while he retained his keen interest in clinical work, he had already made up his mind to go forward for Anatomy and he became, first, a Demonstrator under Howden, and later a Lecturer in Anatomy under Stibbe and under the present occupant of the Chair, Professor R. B. Green. In 1943 he was appointed a Reader in Anatomy at Guy's Hospital Medical School in the University of London, and in 1948 he succeeded to the Chair on the retirement of Professor T. B. Johnston.

Coming to Guy's as a trained anatomist, he soon settled down, and it was not long before he established himself on a friendly footing with his students and with his colleagues, but he had few opportunities of showing his administrative ability until the outbreak of the Second World War in September 1939.

Prior to the war it had been provisionally arranged that the Guy's Medical School would in that event move to Oxford, but Whillis and his colleagues felt that the economic results would have inflicted serious hardship on the students and they soon put forward an alternative proposal which was adopted. Having acquired a large, but fortunately empty, Nursing Home on the outskirts of Tunbridge Wells as the headquarters of the School, they proceeded to move everything that was movable from Guy's. Into this work Whillis threw himself heart and soul. Benches, tables, cupboards, bookcases, equipment and domestic fittings were loaded into lorries returning empty from the London markets to the fruit and vegetable gardens of Kent. Interviews with billeting officers followed and showed the necessity for making special arrangements for the housing of students. And so, three or four large private residences, evacuated by their owners, were found, additional beds, etc., were purchased, and arrangements for the housing of some 250 students were completed. In this way the Guy's Medical School was converted into a Residential University Institution. It had to be seen to be believed, for in six weeks it had been established and was continuing its teaching, up to University standards. For nearly five years it continued to function, and during that time Whillis and others of his colleagues, with the help of their wives, continued to conduct it. It was in practice a cross between the ordinary public school and a residential University. During the whole of this time Whillis gave everything he had to give. His teaching retained its conspicuous clarity, and nothing was allowed to interfere with the ordinary running

of the Dissecting Room or with the periodical departmental examinations of dissectors. It was a great piece of organization, and an enormous amount of work inevitably fell on his own shoulders. In addition, there were National duties which he willingly undertook, and the training of students for the Home Guard was a serious item.

The return to Guy's in the early summer of 1944 eased the strain a little. It soon became apparent that his absence in the wilderness for so long had developed and strengthened his character and his self-confidence and he came back, tired, but a new man. Soon he was able to renew his active interest in some of the problems of the living body by which he had always been attracted. His intimate contacts with his clinical colleagues provided him with many opportunities to study the movements involved in swallowing and in speech production. He made many cinematographic records, some of which have already been published, and he succeeded in working out the parts played by the tongue, the soft palate and the pharyngeal muscles. It was this inherent interest in the living body that drew him to a special study of the practical aspects of proprioception and to the whole problem of rehabilitation. There can be little doubt that he was right in his thesis that many patients were returned as fit for duty on insufficient grounds and were regarded as malingerers if they failed, as they often did, to come up to expectations. Just before his final illness he had devised a prosthesis for cases of upper limb amputations, which depended for its proper functioning on the electrical currents generated in muscle. He had promised to show this prosthesis to the Summer Meeting of the British Orthopaedic Association in 1954, but, when his illness intervened, his colleagues and co-workers were able to give the demonstration and it aroused considerable interest and discussion.

For several years he had paid regular monthly visits to the Ministry of Pensions Hospital at Stoke Mandeville and to the Plastic Surgery Unit at East Grinstead Hospital, where he was ever welcome. Mr T. P. Kilner wrote of these visits in the *British Medical Journal*: 'These visits were to us a means of keeping us on the right lines anatomically and functionally and to him a link with the clinical applications of his speciality...and his presence was as invigorating as a breath of fresh air. If time allowed, he would round off the session with a "lecturette" on some anatomical subject of our choice, before we carried him off to dine with us at Aylesbury.'

In 1950 he accepted the invitation of the Council of the Chartered Society of Physiotherapists to become its Chairman, and in this capacity he played a very active part in connexion with the discussions which arose out of the Cope Report. He was particularly interested in the requirements for recognition, and fought hard, and not without success, for a reasonably high standard at all stages. In 1953, when the Inaugural Congress of the World Confederation for Physical Therapy was held in Central Hall, Westminster, he was unanimously elected to be Chairman and he took an important part both in the discussions and in the social activities of the Congress.

Soon after he succeeded to the Chair at Guy's, the School invited him to become the Director of the Department of Medical Illustration and this gave him new scope for the exercise of his administrative ability. Without encroaching in any way on his anatomical work, he succeeded in reorganizing a complicated department with its



JAMES WHILLIS, M.D., M.S. F.R.C.S.

*(Facing p. 420)*





own peculiar problems and in raising it to its present high standard of work. Further, he was able to suggest the lines along which it should develop so as to provide for the requirements of the Hospital and the School and to play its part in research.

In addition to contributing a number of papers to this and to other journals, Whillis published in 1938 an *Elementary Anatomy and Physiology*, now in its third edition, and, in collaboration with Professor Lucas Keene, he wrote an *Anatomy for Dental Students*, in which, amongst other original items, he published a series of figures of unusual dissections of the mouth from the inside, which he had personally carried out and which had very material teaching value. From 1942 onwards he acted as a much-valued co-editor of *Gray's Anatomy* and, before his final illness overtook him, he had completed his share of the work for the recently published 31st edition. His influence can be recognized in the character and finish which stamps many of the new figures and the conspicuous clarity of those parts of the text for which he was responsible.

Whillis was *persona grata* wherever he went. As a teacher he had no difficulty in making the spoken word clear and he had the gift of making rapid and accurate drawings on the blackboard. He was an able and a popular teacher and many tributes have come to Guy's from his past students, who rarely omitted to single out his skilful draughtsmanship and his never failing courtesy and approachability for special mention. His department always ran smoothly, and his staff knew they could rely on him for advice and, when necessary, for support, for he took a keen interest in everything affecting their welfare. He was always on good terms with his colleagues in other departments and appreciated the value of give and take. When occasion arose they never hesitated to seek his opinion or his advice, for his motives were above suspicion and were never influenced by self-interest. Always open and frank, he could and did speak his mind with no uncertainty when the occasion arose but he was so transparently honest and sincere that he made no enemies. A loyal and helpful colleague, he was free from all prejudice and ungrudging in his praise of others. His outside interests were many and his fondness for things of beauty was shown by his wide knowledge of old furniture. When the opportunity offered, he loved a game of golf and, on his day, he was no mean opponent. He was by nature a quick thinker but it did not take him long to appreciate the value of second thoughts, so that his judgement was always sound and reliable. By his untimely death British Anatomy has lost one of its most active brains and Guy's one of the most loyal of her adopted sons.

T.B.J.

## REVIEWS

*Prefrontal Leucotomy and Related Operations: Anatomical Aspects of Success and Failure.* By A. MEYER and ELIZABETH BECK. (60 pp., 20 figures;  $9\frac{3}{4} \times 7\frac{1}{2}$  in.; 10s. 6d.) Edinburgh, published for the William Ramsay Henderson Trust by Oliver and Boyd 1954.

This work, which is based on Prof. Meyer's William Ramsay Henderson Lecture, is of the nature of a preliminary report on the neurological findings in 102 cases, all of whom had survived a 'blind' leucotomy operation by more than 5 months. Its main importance lies in the attempt which is made to relate the anatomical location and extent of the lesion with the clinical results of the operation. For the purpose of localization five coronal planes are defined (anterior, mid-anterior, middle, mid-posterior and posterior) and their level in the frontal lobe marked on a chart showing Brodmann's areas. Each plane is divided into seven segments, dorso-medial, dorso-lateral, mid-lateral, etc.; the lesion is then described with reference to a particular plane and to the segments of that plane. It is clear, of course, that localization in such terms cannot be very precise, although in material of the kind available and for the purpose in view it is probably the most suitable. More detailed investigations of the nature and results of the lesion (so far as degeneration of fibres and cells is concerned), having been published elsewhere, are referred to only in a short but useful summary of the afferent and efferent connexions of the frontal lobe.

In the attempt to establish a relationship between the extent and location of the lesions and the degree of improvement or deterioration in classified groups of psychiatric cases a statistical method is used. The analysis is of a preliminary nature, and more detailed statistical investigations on the material are said to be in progress. At present all that can be established appears to be that 'quantitative adequacy' of the cut is related to improvement or recovery, and that lesions in the dorsal and dorso-lateral segments of the white matter in the frontal lobe are not as significant for improvement as those in the mid-central, orbital and cingulate segments.

In general the book will be of chief interest to psychiatrists and surgeons. Anatomists interested in the relation between structure and function in the cerebral hemispheres will find it a short and clear account of work in a particularly difficult field. While the conclusions which can at present be based on this work may seem somewhat meagre, one must nevertheless congratulate the authors and their co-workers for their enterprise in seizing the opportunity offered by the present frequency, and in some cases lethal effects, of surgical interference with the frontal lobes.

F. GOLDBY

*The Biochemistry of Semen.* By T. MANN. Methuen's Monographs on Biochemical Subjects. (Pp. v+240; 16s.) Methuen and Co. Ltd. 1954.

With the ever-increasing attention paid to the problems of fertility, artificial insemination and sperm storage, the monograph fulfils a real need. Few are better qualified to satisfy this need than Dr Mann who has himself made substantial contributions in this field. From 1677, when Hamm and Leeuwenhoek discovered the spermatozoon, until the end of the nineteenth century, when Miescher produced his important contributions on the milt of the salmon, attention was focused almost exclusively on the spermatozoon, and the liquor seminis was considered merely as a vehicle in which active spermatozoa were suspended. Even the spermatozoa were the subject of the widest speculation. They were often classified with the Infusoria and the Helminthi and characterized by a complicated organization, intestines, gastric sacculi and even generative organs. Owing to technical difficulties the



serious study of mammalian seminal fluid had to wait until this century, and the present monograph marks the first comprehensive publication of the accumulated biochemical knowledge, particularly concerning the mammals.

This book will undoubtedly become the standard source of information concerning semen. As its title suggests it is almost entirely devoted to the biochemistry of semen, spermatozoa and seminal plasma. Morphological details are necessarily brief but adequate. It is written with a clarity which can only come from one with a complete familiarity with the subject, and should be readily understood by those with but a limited knowledge of biochemistry. It deals with the properties and composition of whole semen, the spermatozoa and seminal plasma, the proteins, enzymic systems, lipids, spermine, choline, ergothioneine, creatine, creatinine, citric acid, fructose and other substances, together with their probable physiological roles. The account of fructose and fructolysis, a field in which Dr Mann himself has made notable discoveries, should be of particular interest to embryologists.

The monograph should be of considerable value to workers not only in the reproductive field, but also to those in veterinary science in general and in forensic medicine. There is here also a mass of basic information awaiting the attention of the histochemist. The book is singularly free from errors and is provided with a very full list of references.

D. V. DAVIES

*Vertebrate Dissection.* By WARREN F. WALKER, JUN., Ph.D. (Pp. ix+331; 62 illustrations; 17s. 6d.). Philadelphia and London: W. B. Saunders Co. 1954.

The author's purpose, as stated in the preface, is to assist the student to carry out a reasonably thorough examination of a few animals 'with the view of making the anatomy of the mammal meaningful'. This purpose is achieved. The systemic plan is used and the animals chosen are the dogfish (*Squalus*), the mudpuppy (*Necturus*), and the cat and/or rabbit: instructions are also given for the study of a balanoglossid, an ascidian, *Amphioxus*, the lamprey and the *Ammocoetes* larva.

The book is effectively devised and arranged to encourage the student to approach the practical study of animal structure with intelligence instead of merely as a skilled craftsman; certainly anyone who conscientiously worked through this book would have no one but himself to blame if he had not at the end acquired a very good basic knowledge of vertebrate anatomy in an interesting way. Good examples of the method of approach are seen in the sections on the skull and the circulatory system. The information given is accurate, up to date and clearly written. Figures are mostly borrowed and are mainly chosen to enlighten the more general aspects of the subject rather than to illustrate specific practical points. The production is good and typographical errors are few. The instructions for dissection are essentially practical and show appreciation of some of the student's difficulties; they are printed in a heavy black type which is cumbersome at first sight but which in fact is quite comfortable to read and is easily seen while dissecting.

The only serious criticism which might be made is the relative neglect of the peripheral nervous system. The cranial nerves of the mammal are not dissected (except in so far as they are seen on the isolated sheep's brain) and are dismissed on page 185 with the words 'the peripheral distribution and composition of the nerves is, with a few exceptions, essentially the same as in fishes'. (Incidentally examination of the sheep's brain instead of that of the cat or rabbit means that the cranial cavity of the animal which is being dissected is not examined in the fresh state which seems a pity as it would help to reinforce and 'make more meaningful' the study of the dried skull.) Similarly, the peripheral spinal nerves are not dissected except for the brachial plexus and then no serious attempt is made to follow the nerves to their termination in the limb; although there may not be room for this, it would seem desirable to indicate the importance of the nerve supply when considering the homologies of muscles as on pages 122-3.

In the next edition it is to be hoped that, when referring to the interventricular foramen, the name *Monro* will be correctly spelt (pp. 178, 192 and index) and that the *Harderian*

gland will be given a capital initial letter (p. 148); postcaval should read precaval (p. 262, twice); the ligamentum arteriosus is thus miscalled on pages 266, 269 and in the index; the synotic tectum is referred to as 'synoptic' on pages 40, 55 and in Fig. 7. It would be useful to have a list of figures and tables, there is none at present, nor, when any of these is mentioned in the text, is there any reference to the page on which it will be found.

These are small points. It is refreshing to find a book of this introductory type which shows both a stamp of originality and evidence of very careful writing. It was a pleasure to read and it is strongly to be recommended.

H. L. H. H. GREEN

*Cytoarchitecture of the Human Brain Stem.* By J. OLSZEWSKI and D. BAXTER. Basel and New York: S. Karger. 1954. (Swiss francs 72.80.)

This volume from the Montreal Neurological Institute is concerned principally with the cellular structure and arrangement of the reticular formation, but includes full accounts of the cytoarchitectonics of the cranial nerve nuclei, the relay nuclei of the brain stem, including the superior colliculus, and a large number of nuclei of unknown or ill-known connexions. Useful bibliographies are given for each section. The reproductions of microphotographs are remarkably good and the general production of the volume is excellent.

This beautifully illustrated atlas is deserving of a place in every anatomical library.

J. D. BOYD

## BOOKS RECEIVED

*Grundriss der allgemeinen Zoologie.* By Alfred KUHN. Tubingen: 11., verbesserte Auflage, 1955. (Pp. viii+281; 223 illustrations; Ganzleinen. D.M. 16.50.) Stuttgart: Georg Thieme Verlag.

*Shearer's Manual of Human Dissection.* Edited by C. E. TOBIN, 3rd edition, 1955. (Pp. xi+287; 79 illustrations; 49s. New York: The Blackiston Division, McGraw-Hill Book Co.

*Maxillofacial Anatomy, with Practical Applications.* By H. SHAPIRO, (Pp. xiv+392; 314 illustrations, 46 in colour; 96s.) Philadelphia: J. P. Lippincott Co. 1954.

*Human Limbs and their Substitutes.* By P. E. KLOPSTEG and P. D. WILSON. Prepared under the sponsorship of the Advisory Committee on Artificial Limbs, National Academy of Sciences National Research Council. (Pp. xiv+844; 96s.) New York: McGraw-Hill Book Co. 1954.

*Rauber-Kopsch Lehrbuch und Atlas der Anatomie des Menschen.* Edited by FR. KOPSCH, Berlin, 19., durchgesehene und verbesserte Auflage, in zwei Bänden. Band I: Allgemeines, Skeletsystem, Muskelsystem, Gefässsystem, 1955. (Pp. vii+736; 731 illustrations, many in colour; Ganzleinen. D.M. 64.50.) Band II: Eingeweide, Nervensystem, Sinnesorgane, 1955. (Pp. viii+752; 782 illustrations, many in colour; Ganzleinen, D.M. 64.50.)

# ESTERASE ACTIVITY IN THE SKIN OF MAMMALS\*

BY WILLIAM MONTAGNA AND VICTOR R. FORMISANO

*Arnold Biological Laboratory, Brown University, Providence 12, R.I.*

## INTRODUCTION

Thorough histochemical studies of the distribution of esterases have been made of practically all of the organs except the skin. Kung (1949) has written the only paper dealing with esterases in the skin of the mouse. In this paper we present a report of the distribution of esterases in the mouse, rat, guinea-pig, rabbit, cat, ox and pig. We have used the technique of Nachlas & Seligman (1949), which, when handled with care, gives good histological results. The technique appears to be sensitive and the enzyme sturdy enough to resist formaldehyde fixation. It will be emphasized that there are striking species differences in the amounts and distribution of the enzyme in the skin of the animals studied. Also, in the skin of the mouse and rat the amount of the enzyme fluctuates with the cycles of hair growth.

## MATERIALS AND METHODS

Pieces of skin from the backs of six white mice and six white rats were removed from regions which contained resting hair and regions with growing hair. Skin was also obtained from the back and head of two hairless mice, six white guinea-pigs, and from the backs and ears of six cats, and two rabbits. Relatively fresh skin from two steers and two pigs was collected in a slaughter house.

The tissues were fixed 24 hr. in chilled 10 % neutral formaldehyde, since acetone distorts them and shows no advantages in preserving the enzyme. In practice, the tissues may be kept in the fixative for several days without impairing enzymic activity. The tissues were cut with freezing microtome at 15 $\mu$ , and the sections were hardened in 10 % formalin for 2 hr.; the sections were then carried in a Gooch crucible for 5 min. through each of the following solutions: (a) equal parts of acetone and 10 % formaldehyde, (b) absolute acetone, and (c) equal parts of acetone and distilled water. After rinsing in three changes of distilled water, the tissues were incubated for 5–15 min., at room temperature, in the incubation mixture of Nachlas & Seligman (1949), as modified by Gomori (1952*a*). The medium was prepared by first dissolving 20 mg.  $\alpha$ -naphthyl acetate in 0.25 ml. acetone; after adding 20 ml. 0.01 M phosphate buffer, adjusted to pH 7.4, the solution was agitated until some of the initial cloudiness disappeared, and then 20 mg. naphthanil diazo blue B was added to it. The mixture was filtered before use.

After incubation, the sections were washed in distilled water and mounted on slides in glycerine jelly. The preparations keep well for months at room temperature. A grey, blue or purple colour indicates enzyme activity. Control sections which had been exposed to hot steam for 5 min. or sections incubated in the mixture without the  $\alpha$ -naphthyl acetate showed no reaction.

\* This work was supported in part by a grant from the United States Public Health Service RG 2125 C4.



## OBSERVATIONS

In the skin of most animals studied the epidermis shows some enzymic reaction, but it is most abundant in the epidermis of the rat (Pl. 3, fig. 10) and least abundant in that of the hairless mouse (Pl. 3, fig. 8) and the guinea-pig. In general, the cells in the lower Malpighian layer are more strongly reactive than those further up. The cells in the stratum granulosum and those in the stratum corneum are usually unreactive. In the rabbit and the cat, the cells of the Malpighian layer have only a small amount of enzyme, but a band of cells between the stratum granulosum and the stratum corneum is very strongly reactive (Pl. 1, fig. 1). In the epidermis of man, a comparable esterase-rich band is exceptionally well delineated (Montagna, 1955).

Sebaceous glands are richest in enzyme activity in the skin of the cat (Pl. 2, fig. 5), the rat (Pl. 3, fig. 10), and the mouse (Pl. 1, figs. 2, 3, Pl. 4, figs. 12, 13), and poorest in the rabbit (Pl. 3, fig. 9), the guinea-pig (Pl. 3, fig. 11), pig and ox. In the mouse and rat the entire gland, including the newly formed sebum, but not the sebum in the ducts, is strongly reactive (Pl. 2, fig. 3). When the hair follicles are quiescent, the sebaceous glands in the mouse and rat are very strongly reactive and the glands are sharply outlined by the reaction (Pl. 1, figs. 2, 3; Pl. 3, fig. 10; Pl. 4, fig. 12). When the hair follicles are growing, however, the glands are so strongly reactive that the enzyme or the dye indicating activity diffuses or spills into the surrounding connective tissue and the outlines of the glands are no longer clear-cut (Pl. 4, fig. 13). In the sebaceous glands of the cat, where the reaction is stronger at the periphery of the gland than in the centre, there is always some diffusion of the dye in the connective tissue around the glands, whether the glands are associated with growing or with resting hair follicles. In the glands of the guinea-pig, the rabbit, the ox and the pig the reaction is weak or absent.

The skin of hairless mice is unique in possessing in the dermis numerous sebaceous and corneal cysts (Montagna, Chase & Melaragno, 1952). The sebaceous cysts have strong enzyme reaction, but the corneal cysts are unreactive. Sebaceous glands are always strongly reactive, even when they are adventitious buds growing from the walls of corneal cysts (Pl. 3, fig. 8).

The 'ceruminous' glands in the ears of the cat are moderately to strongly reactive (Pl. 2, fig. 5). The glands in the sole and digital pads of the cat and the rat are weakly reactive or unreactive. In the skin between the horns of the ox, there are two groups of sweat glands. One group, more numerous and found deeper in the dermis, is lined by moderately esterase-reactive, small cuboidal cells (Pl. 2, fig. 7). Other glands, found at higher levels in the dermis, are lined with strongly reactive, large columnar cells riddled with vacuoles (Pl. 2, fig. 6). In the pig, the apocrine glands are more numerous in the head than elsewhere. They are lined with small, moderately to weakly reactive cuboidal cells with a few strongly reactive cells scattered among them.

All hair follicles show some enzyme reaction. In the mouse, rat and cat the hair follicles have much stronger enzyme reaction than those of the guinea-pig and rabbit. In resting hair follicles the epithelial sleeve which surrounds the hair club and the so-called 'hair germ', as well as the dermal papilla, are moderately reactive. In active hair follicles, the outer sheath shows stronger enzyme reaction. The lower

half of each follicle is always richer in esterase activity than the upper half, and the lower part of the bulb has the strongest reaction of all. The dermal papilla is usually strongly reactive, particularly in the mouse, rat (Pl. 1, fig. 4) and cat.

In the mouse, rat and cat, the small blood vessels in the dermis have a strongly reactive tunica intima. In the other animals it is less reactive. The adipose layer is particularly reactive in the mouse, rat, cat and pig but weakly reactive in the rabbit, guinea-pig, and ox. The arrectores pilorum muscles are moderately reactive and the fibres of the panniculus carnosus are strongly reactive.

#### DISCUSSION

With the Tween technique of Gomori (1952*b*) esterase activity can be demonstrated in the skin of some mammals but not in that of others. For instance, the sebaceous glands of the hamster (Montagna & Hamilton, 1949), mouse (Kung, 1949) and the rat are strongly reactive, but those of man, the guinea-pig and rabbit show practically no reaction. The technique of Nachlas & Seligman (1949) also reveals species differences, but the esterases revealed by the two techniques are not necessarily the same enzymes (Gomori, 1952*a*). The presence or absence of non-specific esterases is not the only instance of such species differences; it is well known that although the sebaceous glands of the rat and cat are rich in alkaline phosphatase, those of the mouse, hamster, guinea-pig and man show only traces of reaction. It is hard to explain such species differences since it is not known precisely what these enzymes do in tissues. Non-specific esterases might be related to the skin surface lipids. These lipids contain large quantities of free fatty acids, which could be released, from the hydrolysis of esters of short and long-chained fatty acids with various alcohols (cf. Nachlas & Seligman, 1949). Skin surface lipids show pronounced chemical differences in different species (Rothman, 1954), and these differences may be reflected in the different amounts and types of esterases in the skin. Other species differences such as presence of small or large amounts of esterase activity in the outer sheath of hair follicles cannot be explained on this basis.

In the mouse and in the rat, where the hair follicles grow in waves, the increase and decrease of esterase which accompany hair growth cycles provide another example of what Strauss & Kligman (1954) call skin cycles. During the periods of growth and rest of hair follicle, the entire skin undergoes profound changes in thickness (Chase, Montagna & Malone, 1953). Together with these changes the skin responds differently to irritating agents (Chase & Montagna, 1951) depending on whether the hair is growing or is resting. Skin is morphologically and physiologically different under these conditions. It is perhaps significant that the greatest overall amount of non-specific esterases is found during the period of hair proliferation.

With the exception of those in the Catarrhines, the anthropoid apes and man, the majority of the sweat glands of mammals are considered to be apocrine glands. Eccrine and apocrine sweat glands are profoundly different organs. They develop differently in the embryo, their *morphology* and physiology are different and they show *differences* in the content of enzymes. In the skin of man, for instance, apocrine glands abound in esterase activity but eccrine glands do not (Montagna, 1955). The eccrine glands in the feet of mice, rats and cats show practically no esterase activity,

whereas the ceruminous glands of the cat and the body sweat glands of the pig and the ox are rich in the enzyme. In the skin of these animals, then, eccrine and apocrine glands can be clearly separated according to the amount of esterase activity in them.

#### SUMMARY

1. The epidermis in all of the skin studied has at least some non-specific esterase activity. In the mouse and rat the enzyme is present in the entire Malpighian layer, and the reaction fades gradually in the upper layers. In the epidermis of the cat and the rabbit, the Malpighian layer is weakly reactive but an esterase-rich band occupies a region comparable to the stratum lucidum.

2. The sebaceous glands are strongly reactive in the rat, mouse and cat but weakly reactive or negative in the guinea-pig, rabbit and ox. The reaction is strongest when the associated hair follicles are growing.

3. Growing hair follicles in the mouse, rat, cat and pig have more enzyme activity than resting follicles. The hair follicles of the guinea-pig and rabbit have very little demonstrable enzyme, regardless of the hair growth cycle.

4. The ceruminous glands of the cat and certain tubular glands in the skin of the ox are rich in esterase activity. Other tubular glands in the deeper part of the skin of the ox and in the pig show moderate reaction. Strong enzyme reaction is a characteristic feature of apocrine sweat glands.

#### REFERENCES

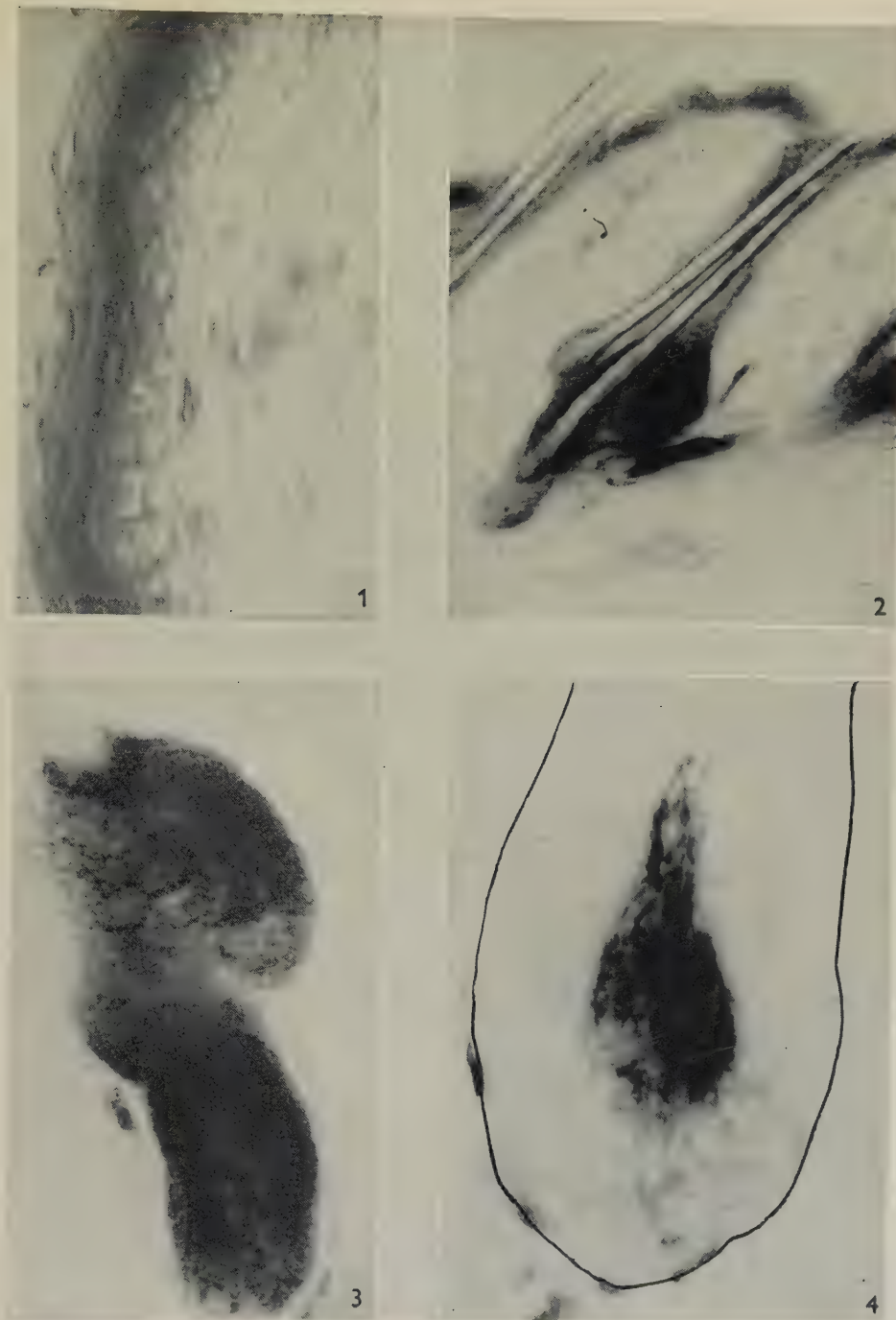
- CHASE, H. B. & MONTAGNA, W. (1951). Relation of hair proliferation to damage induced in the mouse skin. *Proc. Soc. exp. Biol., N.Y.*, **76**, 35-37.
- CHASE, H. B., MONTAGNA, W. & MALONE, J. B. (1953). Changes in the skin in relation to the hair growth cycle. *Anat. Rec.* **116**, 75-81.
- GOMORI, G. (1952a). *Microscopic Histochemistry. Principles and Practice*. Chicago: University of Chicago Press.
- GOMORI, G. (1952b). The histochemistry of esterases. *Int. Rev. Cytol.* **1**, 323-335.
- KUNG, S. K. (1949). Lipase activity during experimental epidermal carcinogenesis. *J. Nat. Cancer Inst.* **9**, 435-438.
- MONTAGNA, W. (1955). Histology and cytochemistry of human skin. IX. The distribution of non-specific esterases. *J. Biophys. Biochem. Cytol.* **1**, 13-16.
- MONTAGNA, W., CHASE, H. B. & MELARAGNO, H. P. (1952). The skin of hairless mice. I. The formation of cysts and the distribution of lipids. *J. invest. Derm.* **19**, 83-94.
- MONTAGNA, W. & HAMILTON, J. B. (1949). The sebaceous gland of the hamster. II. Some cytochemical studies in normal and experimental animals. *Amer. J. Anat.* **84**, 365-388.
- NACHLAS, M. M. & SELIGMAN, A. M. (1949). The histochemical demonstration of esterase. *J. Nat. Cancer Inst.* **9**, 415-425.
- ROTHMAN, S. (1954). *Physiology and Biochemistry of the Skin*. Chicago: University of Chicago Press.
- STRAUSS, R. E. & KLIGMAN, A. M. (1954). The effect of mitotic poisons on hair growth in mice. *J. invest. Derm.* **22**, 515-519.

#### EXPLANATION OF PLATES

##### PLATE 1

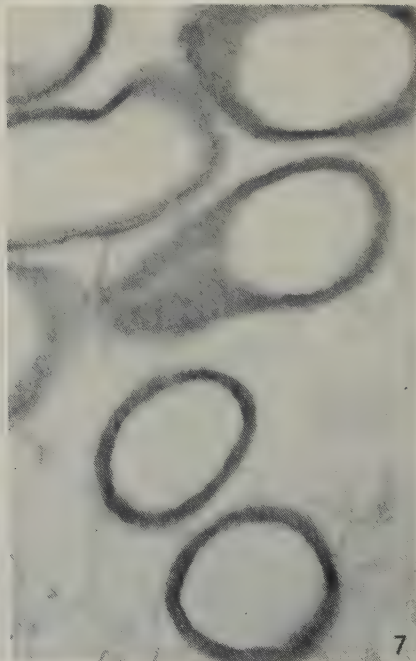
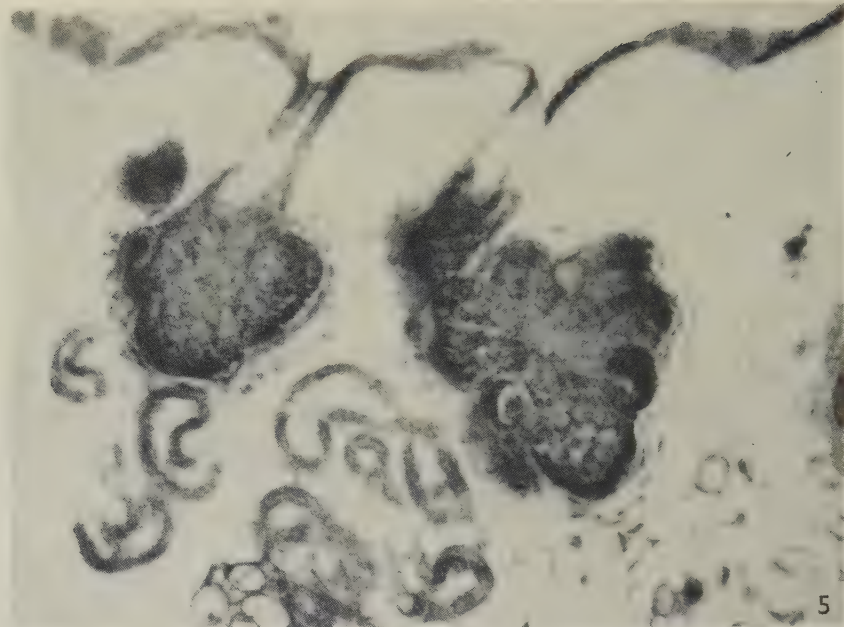
- Fig. 1. Epidermis of the rabbit showing an esterase-rich band between the weakly reactive Malpighian layer and the negative corneal layer.  $\times 525$ .
- Fig. 2. Resting hair follicle of the mouse, containing two club hairs. The entire epithelial portion of the follicle is reactive; the sebaceous gland is strongly reactive.  $\times 125$ .
- Fig. 3. Strongly reactive sebaceous gland from the skin of the hairless mouse. The sebum in the duct is unreactive.  $\times 525$ .

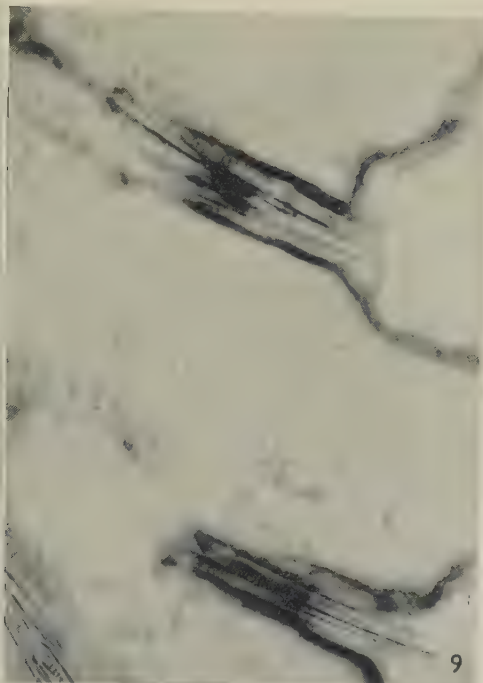
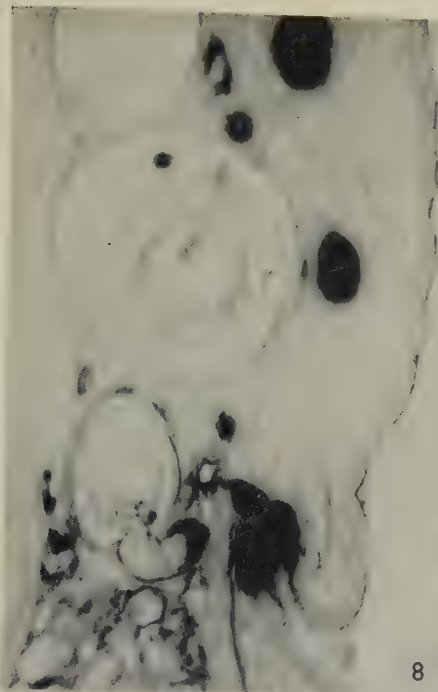




MONTAGNA AND FORMISANO—ESTERASE ACTIVITY IN THE SKIN OF MAMMALS

(Facing p. 428)







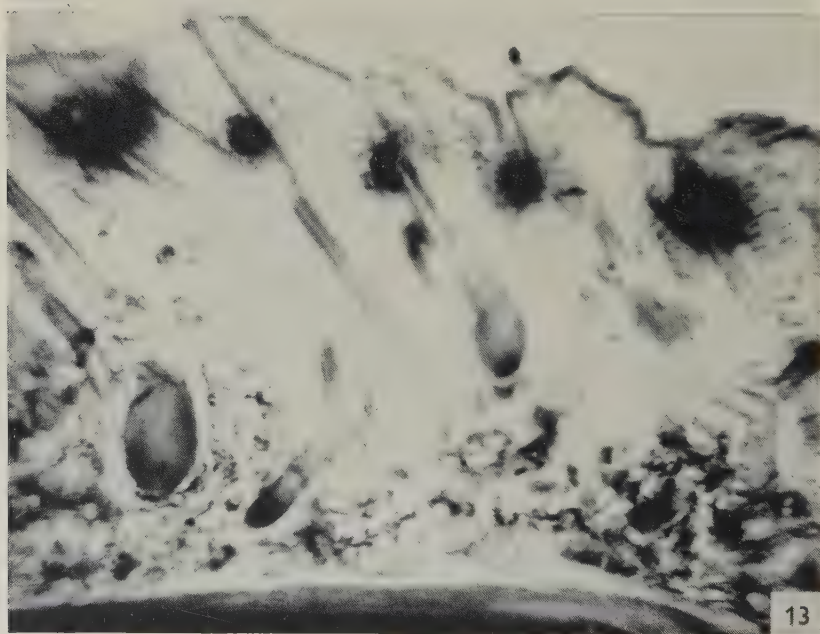


Fig. 4. The bulb of a hair follicle of the rat with its outer periphery outlined in ink. This section was deliberately under-incubated in the substrate so that only the strongly reactive dermal papilla would show up to good advantage. In this section the papilla appears like an island, although it is continuous with the connective tissue sheath.  $\times 525$ .

PLATE 2

Fig. 5. Sections through the ear of the cat. The epidermis sebaceous glands and ceruminous glands are rich in enzyme activity.  $\times 125$ .

Fig. 6. Large, 'active' sweat glands in the skin of the ox.  $\times 125$ .

Fig. 7. 'Inactive' sweat glands in the skin of the ox. From the same section as fig. 6.  $\times 125$ .

PLATE 3

Fig. 8. Skin of the hairless mouse. The epidermis and the corneal cysts are weakly reactive. The sebaceous glands and the sebaceous appendages of the cysts are rich in esterase activity.  $\times 125$ .

Fig. 9. Skin of the rabbit with resting hair follicles. Moderate enzyme activity in the epidermis and in the epidermal part of resting hair follicles. The sebaceous glands are practically unreactive and cannot be seen well.  $\times 125$ .

Fig. 10. Skin of the rat, containing resting hair follicles. The entire Malpighian layer of the epidermis is reactive. The epithelial part of hair follicles is moderately reactive and the sebaceous glands show strong activity.  $\times 125$ .

Fig. 11. Resting hair follicle in the skin of the guinea-pig. The epithelial elements of the follicles are weakly reactive. The sebaceous glands are barely reactive.  $\times 125$ .

PLATE 4

Figs 12, 13. Skin of the mouse with resting (12) and with active (13) hair follicles removed from the same mouse. Observe increased esterase activity in the sebaceous glands, hair follicles, and adipose layer in the skin with growing hair follicles.  $\times 60$ .

## A HISTOCHEMICAL STUDY OF THE SEMINAL VESICLE OF THE SHEEP

By R. N. C. AITKEN

*University of Glasgow Veterinary School*

### INTRODUCTION

It has been shown that the reducing sugar present in seminal plasma is fructose and that in some species including the domestic ruminants the principle source of this sugar is the seminal vesicle (Mann, 1946). Mann and his associates have also demonstrated the probable origin of seminal fructose from blood glucose. Thus it has been possible to show fructose production on incubating glucose with minced bull seminal plasma (Mann & Lutwak-Mann, 1948). Again the amount of seminal fructose in rabbits rendered diabetic with alloxan varies directly with the blood glucose concentration (Mann & Parsons, 1950). However, the 6-phospho-fructose from which fructose may be formed by enzymic action (Mann & Lutwak-Mann, 1951) may be produced during the metabolism of both glucose and glycogen, and it is possible therefore that glycogen is formed in the seminal vesicle as an intermediate step in fructose production. Mann, Davies & Humphrey (1949) have shown that fructose production by the seminal vesicle is dependent on the presence of testosterone and that therefore fructose production ceases in the castrate.

Examination of seminal vesicle sections stained by the periodic acid-Schiff technique suggested that glycogen was produced in this gland and that, surprisingly, glycogen was also present in the castrate. It seemed worth while, therefore, to confirm the production of glycogen in the seminal vesicle by histochemical methods and to investigate the possibility that testosterone affects only fructose formation from glycogen and not glycogen production.

Porter & Melampy (1952) and Melampy & Cavazos (1953) have concluded that protein synthesis is a marked feature of the epithelium of the seminal vesicle of the rat, basing their opinion on the finding of a marked degree of cytoplasmic basophilia due to ribonucleic acid (RNA). Wislocki (1949), on the other hand, was unable to demonstrate basophilia in the deer. RNA production in the seminal vesicles of the ram was therefore investigated and comparison made between normal animals and castrates to determine if possible whether any protein production taking place was influenced by androgens.

Specimens from both normal and castrated animals were examined for 'acid' and 'alkaline' phosphatase, as well as for 5-nucleotidase which has been shown to be present in ram seminal plasma (Mann, 1945).

### MATERIAL AND METHODS

The seminal vesicles used in this investigation were obtained from normal and castrate sheep slaughtered at Glasgow abattoir. The age of castrate specimens ranged from approximately 6 months to 3 years. The majority, however, were slaughtered within 1 year to 18 months, and in fact only one animal from which



specimens were obtained exceeded 18 months. Ram lambs are usually castrated within the first 2 months of life and hence the minimum period between castration and slaughter in this material was 4 months and in the majority of cases was in excess of this period. No differences attributable to age were noted. The majority of the normal animals were slaughtered at 4–5 years of age. One animal was, however, approximately 2 years of age and two others between 6 and 8 years old. Again, age differences were not noted. In all, material from thirty-seven animals was examined, thirteen of these being normal and the remainder castrate. Some specimens were from animals slaughtered in the final quarter of the year and some from animals slaughtered in February or March.

*Seminal vesicle used for localization of enzymes*

The gland tissue used for demonstration of enzymes was immersed in a mixture of equal parts absolute alcohol and acetone approximately 20 min. *post mortem*, and was then returned to the laboratory where it was kept in a refrigerator for 24 hr. (Gomori, 1953). The tissue was then dehydrated and embedded in paraffin wax. (Preliminary experiments with similar material placed within 20 min. after death in isopentane cooled in liquid nitrogen and subsequently embedded after drying in an Edwards tissue dryer, show that after chemical fixation there is some loss of 'alkaline' phosphatase activity; nevertheless, the results are qualitatively similar.)

After sectioning, Gomori's calcium phosphate method for 'alkaline' phosphatase was followed, the sections being incubated either in a substrate mixture containing sodium glycerophosphate (a commercial preparation consisting of a mixture of  $\alpha$  and  $\beta$  isomers) adjusted to pH 9–9.5 or in substrate mixture containing adenosine-5-phosphoric acid at pH 8.4 (for 5-nucleotidase) (Gomori, 1953). For 'acid' phosphatase, Gomori's lead method was used with a substrate mixture containing sodium glycerophosphate at pH 5. All reactions were controlled by use of substrate media from which phosphate had been excluded. Sections were incubated for the following periods: 'alkaline' phosphatase 1, 2 and 18 hr.; 'acid' phosphatase 2, 4 and 18 hr.

*Seminal vesicle used for demonstration of lipid*

For this purpose the gland tissue was fixed in formaldehyde-calcium (Baker, 1946) for 24 hr. and then sectioned on the freezing microtome and stained for 1 hr. in a saturated solution of Sudan black B (Gurr) in 70% alcohol. Other tissues fixed in formaldehyde-calcium were used for Baker's acid haematin test for phospholipids (Baker, 1946).

*Seminal vesicle used for demonstration of polysaccharide*

Other portions of seminal vesicle were fixed in corrosive formol or in Zenker's fluid. Sections fixed in the former were stained with Schiff's reagent after oxidation by periodic acid (McManus, 1946; Hotchkiss, 1948*a, b*; Gomori, 1953) for demonstration of polysaccharides containing the 1:2 glycol grouping. Sections were also treated in this way after treatment with saliva and hyalase (Rhondase, Evans—1 mg./100 ml. borate buffer at pH 6.8 for 12 hr.—Kramer & Windrum, 1954). Corrosive fixed specimens were also stained with Southgate's mucicarmine and both

corrosive and Zenker fixed specimens were stained with the methyl green-pyronin method of Unna-Pappenheim and with toluidine blue. The latter was used as a 0.05 % solution at pH 5 and sections were immersed for 30 min. at 37° (Melampy & Cavazos, 1953).

## RESULTS

The seminal vesicle of the normal adult ram is a compound tubular gland in which the individual tubules of each lobule are separated from each other by thin trabeculae of connective tissue (Pl. 1, fig. 1). The gland tubules vary markedly in diameter, and as a rule narrow and wide tubules occur side by side in the same lobule. Sometimes, however, a lobule is found in which the majority of the tubules are of large diameter.

The secretory tubules are lined by a pseudo-stratified columnar epithelium which averages 29  $\mu$  in height (range 13–55  $\mu$ ). The wider tubules are generally lined by a comparatively low epithelium, but this is not constantly the case. Moreover, these tubules are frequently empty, suggesting that the reduction in height of the epithelium is not due to distension of the tubule with secretion. The epithelium usually consists of cells of two types, viz.:

(1) Tall, columnar cells extending from the thin basement membrane to the free surface.

(2) Angular or rounded cells placed between the bases of the columnar cells. These basal cells are not numerous and are scattered singly amongst the columnar cells (Pl. 1, fig. 1).

Rarely does a thin rod-like cell appear amongst the columnar cells. It is doubtful, however, whether this represents a special type of cell.

The cytoplasm of the basal cells is frequently largely unstained in haematoxylin and eosin preparations, and these then appear as a thin envelope of cytoplasm enclosing an apparently empty space in the centre of which is the nucleus. Similar unstained areas are frequent in the proximal parts of the columnar cells (Pl. 1, fig. 2). Attempts to demonstrate lipid in either type of cell by staining with Sudan black B were, however, uniformly unsuccessful.

The free surface of the epithelial cells is very irregular, streamers of cytoplasm extending into the lumen (Pl. 1, fig. 1). These streamers are sometimes expanded at their distal extremities to form cytoplasmic globules, and similar globules may be found in the lumen of such tubules, suggesting an apocrine type of secretion (Pl. 1, fig. 2). The globules contain PAS positive material which may be present in such amounts that they are uniformly stained throughout. In other cases, however, the PAS positive material is present in the form of discrete granules which are clearly embedded in cytoplasm and even where the globule is uniformly PAS positive, treatment with saliva, which removes this material, does not destroy the globules which can still be distinguished clearly. Not all tubules, however, contain such cytoplasmic globules, some containing only a homogeneous mass (Pl. 1, fig. 4) and some containing only a granular material (Pl. 1, fig. 3). Still others contain both granules and cytoplasmic globules (Pl. 1, fig. 1).

PAS positive granules are present in the epithelial cells apart from those found in the cytoplasmic globules (Pl. 1, fig. 3). The number of granules varies in different

cells and when abundant, they may accumulate in the distal cytoplasm, or, rather less frequently, in the proximal cytoplasm close to the basement membrane. In the latter case, rows of granules may be seen on each side of the nucleus, suggesting that the material is produced in the proximal cytoplasm and migrates from there to the distal border.

Secretion is absent from the lumen of most tubules in sections and when present does not completely fill the lumen. (This last may, however, represent a fixation artefact.) This material, when present, is invariably positive with PAS, but the intensity of the reaction is variable. Thus, granular material is often lightly stained, whereas the homogeneous masses frequently encountered stain intensely. Treatment with saliva removes all PAS positive material from the gland cells, but frequently fails to destroy secreted material (Pl. 1, fig. 4). This is particularly true of the homogeneous, intensely PAS positive masses (Pl. 1, fig. 4). This material also resists treatment with hyalase. Moreover, it does not exhibit metachromasia with toluidine blue and is not stained with Sudan black or mucicarmine. It does, however, react strongly with pyronin when stained by the methyl green-pyronin method of Unna-Pappenheim (Pl. 2, fig. 5). The amount of this pyronin positive material is variable and it may exist in the form of large hyaline globules which are sometimes embedded in the epithelium, although apparently not intracellularly. In one such specimen dense clumps of spermatozoa were found in the lumen of some tubules.

In most specimens, whether from normal or castrate animals, the epithelial cells are not basophilic and show no evidence of cytoplasmic RNA when stained with methyl green-pyronin. In a few instances, however, a very thin layer of faintly basophilic material which gives a reaction with pyronin was noted at the free surface. No attempt was made to define this material more accurately by treatment with ribonuclease.

In castrate specimens the gland tubules are markedly reduced in diameter, and this is associated with a very marked increase in the amount of intertubular connective tissue (Pl. 2, fig. 6). There is, moreover, a marked reduction in the size of the gland as a whole. The tubular epithelium is reduced in height, averaging  $17\ \mu$ , (range  $10\text{--}25\ \mu$ ), but this reduction is readily overlooked unless actual measurements are made, because the basal cells apparently increase in number and frequently form a continuous layer beneath the superficial cells (Pl. 2, fig. 7). The superficial cells are, however, reduced in height. Whether the increase in the number of cells in the outermost layer is only apparent and due merely to reduction in the diameter of the tubule or whether it is due to active proliferation resulting in an actual increase in numbers, has not been determined, but there is no evidence of mitosis.

The free surface of the tubular epithelium in castrates is generally even and sharply outlined without cytoplasmic projections (Pl. 2, fig. 7), and in the castrate the vacuolation of the epithelial cells is much more pronounced than in normal animals. In the castrate, however, lipid can be demonstrated on staining frozen sections with Sudan black B, but it is always present in the form of discrete droplets which, although varying considerably in size, are neither large enough nor present in sufficient numbers to account adequately for the degree of vacuolation noted. The lipid droplets are located in the cytoplasm proximal to the nucleus (Pl. 2, fig. 8). Negative results were obtained with Baker's acid haematin test for phospholipids.



The epithelial cells in the castrate contain numerous PAS positive granules which may be found in any part of the cell, though they are usually most abundant in the cytoplasm distal to the nucleus (Pl. 3, fig. 9). This material is removed from the cells by treatment with saliva. The secreted material frequently found in the gland lumen, however, is not removed by treatment with saliva (Pl. 3, fig. 10). Nor does it exhibit metachromasia when stained with toluidine blue or celestine blue and it is negative with Sudan black B and methyl green-pyronin. Usually, however, it gives a distinctly positive reaction with Southgate's mucicarmin. Such mucicarmin positive material is not found in the gland cells.

#### *Normal animals*                      'Acid' and 'alkaline' phosphatases

Variable results are obtained on incubating sections of seminal vesicles in a substrate medium containing glycerophosphate at pH 9-9.8. In some specimens a positive reaction is confined to the basement membrane of the glandular epithelium, the nuclei of basal epithelial cells, vascular endothelium and adjacent connective tissue nuclei. Since connective tissue nuclei at a greater distance are invariably negative, it is probable that a positive reaction in cells adjacent to the basement membrane represents a diffusion artefact. In specimens showing this reaction, the secretion is negative (Pl. 3, fig. 11).

In other specimens from normal animals, however, the reaction is less intense and more widespread, involving all epithelial nuclei and usually the distal cytoplasm. The basement membrane in such specimens may or may not show a positive reaction but if it does, the reaction is usually less intense than in the aforementioned type of reaction and this applies also to the reaction of the vascular endothelium. Secreted material, however, is positive and sometimes strongly so.

When sections of seminal vesicle from a normal animal are incubated in medium containing glycerophosphate and adjusted to pH 5, the reaction is confined to gland cells and affects both cytoplasm and nuclei (Pl. 3, fig. 12). The nuclear reaction is often distinct before a cytoplasmic reaction develops, and the latter sometimes consists of coarse granules developed in the cytoplasm near the distal border of the cell and not immediately adjacent to the nucleus. It is possible, therefore, that the nuclear reaction is not the result of diffusion. After 18 hr. incubation, the reaction is so intense that all cellular detail is obscured and then adjacent connective tissue nuclei may be positive but this is clearly a diffusion artefact.

#### *Castrate animals*

Seminal vesicles from castrates when incubated in substrate medium at pH 9-9.8 are either negative or show a reaction qualitatively similar to the diffuse type of reaction noted in the normal animals, but much less intense.

Seminal vesicles from castrates when incubated in substrate medium adjusted to pH 5 are usually negative or at best show only a slight nuclear response after prolonged incubation (18 hr.).

#### *5-Nucleotidase*

Sections from normal animals incubated in substrate medium containing adenosine-5-phosphoric acid and adjusted to pH 8.5 may be negative, or, with short

incubation periods, may show a reaction qualitatively similar to the diffuse reaction noted in some specimens incubated in glycerophosphate at pH 9-9.5. Only a slight response is obtained, however, after 2 hr., but after 18 hr. incubation there is, in cases which show any reaction at all, a fairly intense but diffuse reaction affecting all structures in the section. Despite the occasional negative result with a similar technique, and despite the fact that controls were negative, the diffuse character of this reaction suggests that it may not be enzymic in origin and in fact with such specimens, an identical response is obtained under conditions likely to preclude enzymic action. Thus a positive reaction is obtained on incubating after immersion of the section in water at 90° for 2-5 min. and after immersion in 1% cyanide for a similar period. The addition of cyanide (1%) to the substrate medium is also without effect. (With castrate specimens the reaction is qualitatively similar but rather less intense.)

#### DISCUSSION

The gland tubules in castrate sheep present the same general features as in the castrate bull (Mann, Davies & Humphrey, 1949). In normal animals, however, there appear to be differences between sheep and bovine seminal vesicles. Thus the above authors identified in bulls a tall, thin columnar cell functionally and topographically associated with the basal epithelial cells and apparently concerned with the transmission of lipid from the latter to the gland lumen. A structurally similar type of cell is rarely found in sheep and apparently bears no relation to the basal cells which, in any event, do not appear to contain lipid. Streamers and globules extending from the free surface as in the ram have been described as occurring in the bull by Mann *et al.* (1949), but these authors do not describe the apocrine type of secretion noted in ram. A reduction in cell height similar to that noted in the castrate sheep has been noted in deer in the non-breeding season (Wislocki, 1949).

The presence in the gland cells of the seminal vesicle of a material which is PAS positive and which is removed by pre-treatment with saliva shows that these cells do produce glycogen. Moreover, variations in the amount of this material in different cells and variations in its location within the cell in similarly treated specimens suggest secretory activity, although only comparatively small amounts of free glycogen can be demonstrated in the lumen of the secretory tubule. It seems probable, therefore, that glycogen is formed as an intermediate stage in fructose production. Whether the small amount of free glycogen which can sometimes be demonstrated in the gland lumen is transformed into fructose or whether it persists as glycogen has not been determined. There is some evidence, however, that the glycogen discharged from the cells enclosed in a cytoplasmic globule is transformed into fructose. Thus the globules in the lumen of a particular tubule sometimes appear to contain less glycogen than do globules still attached to the lining cells of the same tubule, suggesting continued conversion even after separation of the globule from the parent cell.

Fructose production by the seminal vesicle ceases shortly after castration due to absence of testosterone (Mann & Parsons, 1947; Mann *et al.* 1949). Nevertheless, histochemical examination of the seminal vesicles of castrate rams shows that even many months after castration the gland cells contain glycogen in amounts which are approximately equal to the amounts found in the normal gland. Apparently, however,

glycogen is not present in the gland lumen in the castrate, and the free surface of the secretory cells presents a regular appearance contrasting sharply with the irregular surface characteristic of these cells in the normal animal. It is possible, therefore, that testosterone influences the enzymes concerned with the conversion of glycogen to fructose (e.g. the enzymes known to exist in seminal vesicles which dephosphorylate fructose-6-phosphate (Mann & Lutwak-Mann, 1951; Kuhlman, 1954)), and not the production of glycogen by the gland cells. There is, of course, diminished glycogen production in castrates due to a decrease in the total amount of glandular tissue, and activity of individual cells may also be reduced since failure to convert glycogen to fructose may permit its accumulation in the cell and so mask diminished glycogen production. Nevertheless, the basic mechanism is apparently unaffected. In this connexion it is interesting that there is no reduction in the amount of glycogen in glands from normal animals killed during the non-breeding season, although there is then a reduction in the number of testicular interstitial cells and many of those which remain exhibit some degree of atrophy (Maqsood, 1951). In Virginia and Japanese deer glycogen production in the seminal vesicles does appear to be markedly reduced during the non-breeding season (Wislocki, 1949), when there is a reduction in secretion of androgens associated with testicular atrophy. In such species, however, the degree of testicular atrophy is more marked than in sheep, with probably a greater reduction in androgen secretion. There may be, however, a sufficient secretion of androgen to cause some conversion of glycogen to fructose in the seminal vesicle and thereby prevent an accumulation in amounts comparable to those found in the castrate sheep, and therefore there may be no fundamental difference between the seminal vesicles in these two species.

Cytoplasmic basophilia due to RNA is a prominent feature of the rat seminal vesicle (Porter & Melampy, 1952; Melampy & Cavazos, 1953). It is not a marked feature of the ram seminal vesicle, and it is surprising therefore to find in the gland lumina of this species considerable amounts of pyronin positive material. In one case, the presence of this material may be related to the concomitant presence of large masses of spermatozoa.

The PAS positive material sometimes found in the lumina of the tubules of the seminal vesicle in the castrate sheep gives a positive reaction also with Southgate's mucicarmine, but is not metachromatic with toluidine blue or celestin blue and it may be therefore an acid polysaccharide of low acidity (Gomori, 1954).

Lipid is found in the castrate sheep as discrete droplets in the proximal part of the cell. In rats lipid accumulates in a similar situation in castrates (Cavazos, Porter & Melampy, 1954). These authors found a diffuse sudanophilia in the normal animal, but a similar reaction was not noted in the ram. Apparently, however, testosterone influences lipid production and storage in both species. In deer, on the other hand, lipid droplets are present in all parts of the cytoplasm in normal animals (Wislocki, 1949) and in normal bulls lipid accumulates in the basal cells (Mann *et al.* 1949).

The phosphatases which can be demonstrated in tissue sections are inactivated by immersion in water at 90° C. for 2 min. (Danielli, 1953), and some enzymes, including phosphatases, are inactivated by cyanides (Gomori, 1953). It is concluded therefore that the diffuse reaction noted with adenosine-5-phosphate as substrate



at pH 8 is not due to enzymic activity and that therefore 5-nucleotidase is absent from ram seminal vesicles. Whether this applies also to the diffuse reaction sometimes obtained with glycerophosphate at pH 9-9.5 is unknown. Wislocki (1949) did, however, note a similar reaction in deer during the rutting season and a similar reaction had been described for the bull (Rollinson, 1954). Moreover, this type of reaction is comparatively rare in the castrate sheep and when present is always less prominent than in the normal animal, and this also is in agreement with Wislocki's finding that in deer the reaction is much reduced in the non-breeding season. On the other hand, in some normal specimens the reaction was largely stromal. The animals showing this reaction were killed, however, in the final quarter of the year (prior to mid-December) when androgen secretion is presumably maximal, whereas specimens showing the diffuse type of reaction were from animals slaughtered in February and March. There is therefore some evidence in support of the view that the variation in reaction may be associated with variations in androgen secretion. Nevertheless, this is not conclusive, and it is not in accord with the finding that the stromal type of reaction is characteristic of the rat whether castrate or normal, though reduced in intensity in the former (Melampy & Cavazos, 1953; Porter & Melampy, 1952).

The 'acid' phosphatase reaction of the seminal vesicle of the sheep, on the other hand, is intense in normal animals but markedly reduced or absent in the castrate and is clearly under androgenic control. This is supported by findings in deer (Wislocki, 1949) and in rats (Porter & Melampy, 1952). The distribution of 'acid' phosphatase in the normal ram is similar to that described for the bull (Rollinson, 1954).

#### SUMMARY

1. The seminal vesicles of normal and castrate sheep have been examined histochemically.

2. Glycogen has been demonstrated in the secretory cells of normal and castrate animals, and the suggestion made that this substance represents an intermediate stage in the production of fructose. It is suggested also that the presence of glycogen in the gland cells of the castrate indicates that cessation of fructose production by the seminal vesicle after castration is due to failure to convert glycogen to fructose and not to disturbance of the glandular mechanism for converting blood glucose to glycogen. This view is supported by the finding that the amount of glycogen in the gland cells of normal animals is not reduced during the non-breeding season, although there is evidence that in sheep at this time androgen production is reduced.

3. The secretory cells rarely exhibit cytoplasmic basophilia in either normal or castrate animals.

4. Lipid is present only in castrates and its accumulation apparently is related to the secretion of androgens.

5. The distribution of non-specific phosphatase (acid or alkaline) in both castrate and normal animals has been described, and their possible hormonal control discussed. 5-Nucleotidase is absent from the seminal vesicles of sheep.

## REFERENCES

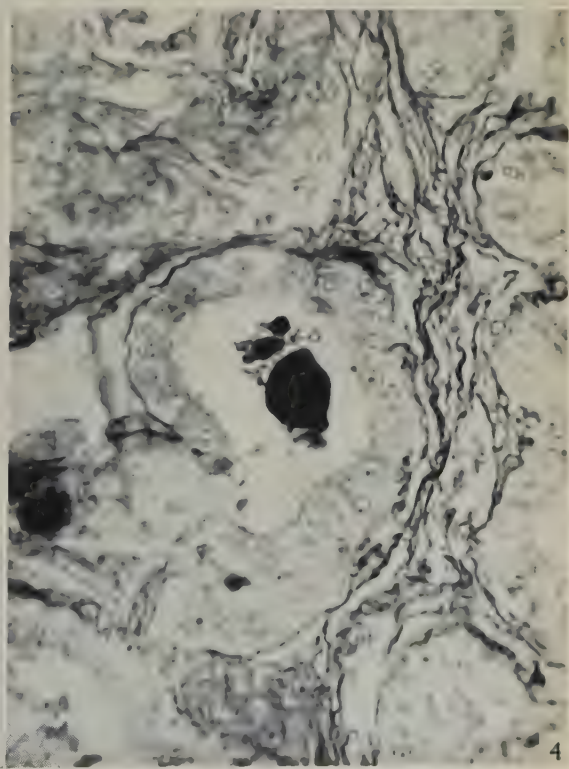
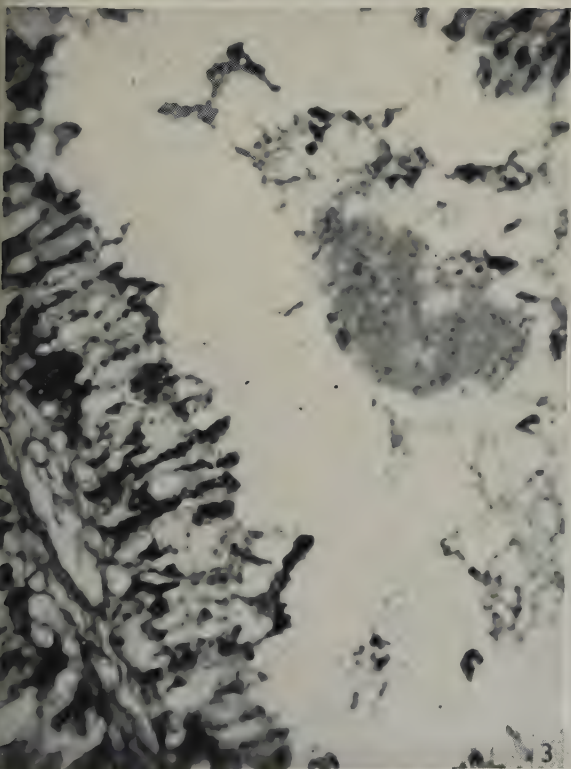
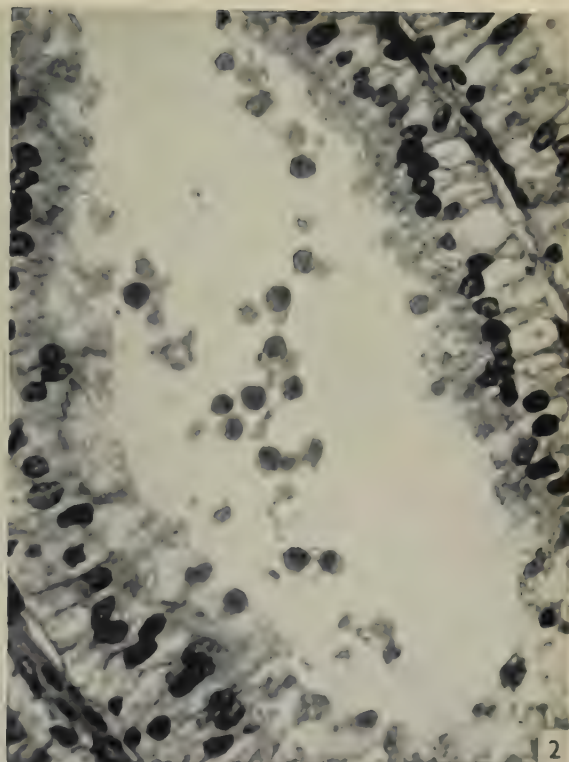
- BAKER, J. R. (1946). The histochemical recognition of lipine. *Quart. J. micr. Sci.* (N.S.), **87**, 441-70.
- CAVAZOS, L. F., PORTER, J. C. & MELAMPY, R. M. (1954). Composition of rat seminal vesicles and effects of testosterone propionate on lipid distribution. *Proc. Soc. exp. Biol.*, N.Y., **85**, 511-515.
- DANIELLI, J. F. (1953). *Cytochemistry. A Critical Approach*. New York: John Wiley; London: Chapman and Hall.
- GOMORI, G. (1953). *Microscopic Histochemistry. Principles and Practice*. University of Chicago Press.
- GOMORI, G. (1954). The histochemical behaviour of acid mucopolysaccharides. *J. Histochem. Cytochem.* **2**, 470.
- HOTCHKISS, R. D. (1948*a*). A microscopical reaction resulting in the staining of polysaccharide structures in fixed tissue preparations. *Arch Biochem.* **16**, 131-141.
- HOTCHKISS, R. D. (1948*b*). Histological and histochemical uses of periodic acid. *Stain Tech.* **23**, 99-108.
- KRAMER, H. & WINDRUM, G. M. (1954). Sulphation techniques in histochemistry with special reference to metachromasia. *J. Histochem. Cytochem.* **2**, 196-208.
- KUHLMAN, R. E. (1954). Hexose-6-phosphatase activity in the seminal vesicles and prostate gland of the rat. *Proc. Soc. exp. Biol.*, N.Y., **85**, 458-460.
- McMANUS, J. F. A. (1946). Histological demonstration of mucin after periodic acid. *Nature, Lond.*, **158**, 202.
- MANN, T. (1945). Studies on the metabolism of semen. 1. General aspects. Occurrence and distribution of cytochrome. Certain enzymes and coenymes. *Biochem. J.* **39**, 451-455.
- MANN, T. (1946). Studies on the metabolism of semen. 3. Fructose as a normal constituent of seminal plasma. Site of formation and function of fructose in semen. *Biochem. J.* **40**, 481-491.
- MANN, T., DAVIES, D. V. & HUMPHREY, G. F. (1949). Fructose and citric acid assay in the secretions of the accessory glands of reproduction as indicator tests of male sex hormone. *J. Endocrin.* **6**, 75-85.
- MANN, T. & LUTWAK-MANN, C. (1948). Studies on the metabolism of semen. 4. Aerobic and anaerobic utilization of fructose by spermatozoa and seminal vesicles. *Biochem. J.* **43**, 266-270.
- MANN, T. & LUTWAK-MANN, C. (1951). Secretory function of male accessory organs of reproduction in mammals. *Physiol. Rev.* **31**, 27-55.
- MANN, T. & PARSONS, U. (1947). Effect of testicular hormone on the formation of seminal fructose. *Nature, Lond.*, **160**, 294.
- MANN, T. & PARSONS, U. (1950). Relationship between testosterone injection in the castrate rat and the fructose and citric acid of the accessory glands. *Biochem. J.* **46**, 440-450.
- MAQSOOD, M. (1951). Seasonal variations in the testis histology of the ram. *Vet. Rec.* **63**, 37.
- MELAMPY, R. M. & CAVAZOS, L. F. (1953). Effects of testosterone propionate on the histochemical reactions of rat seminal vesicles. *Endocrinology*, **52**, 173-187.
- PORTER, J. C. & MELAMPY, R. M. (1952). Effects of testosterone propionate on the seminal vesicles of the rat. *Endocrinology*, **51**, 412-420.
- ROLLINSON, D. H. L. (1954). A study of the distribution of acid and alkaline phosphatase in the genital tract of the zebu bull (*Bos indicus*). *J. agric. Sci.* **45**, 173-178.
- WISLOCKI, G. B. (1949). Seasonal changes in the testes, epididymes and seminal vesicles of deer investigated by histochemical methods. *Endocrinology*, **44**, 167-189.

## EXPLANATION OF PLATES

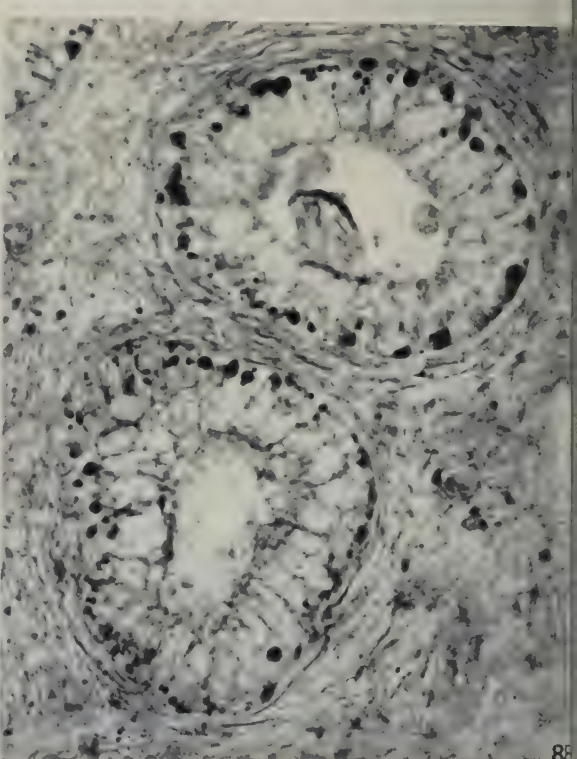
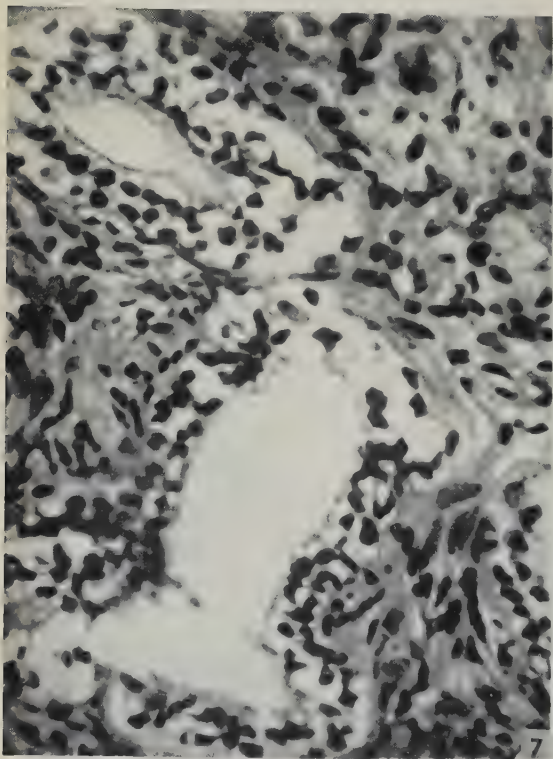
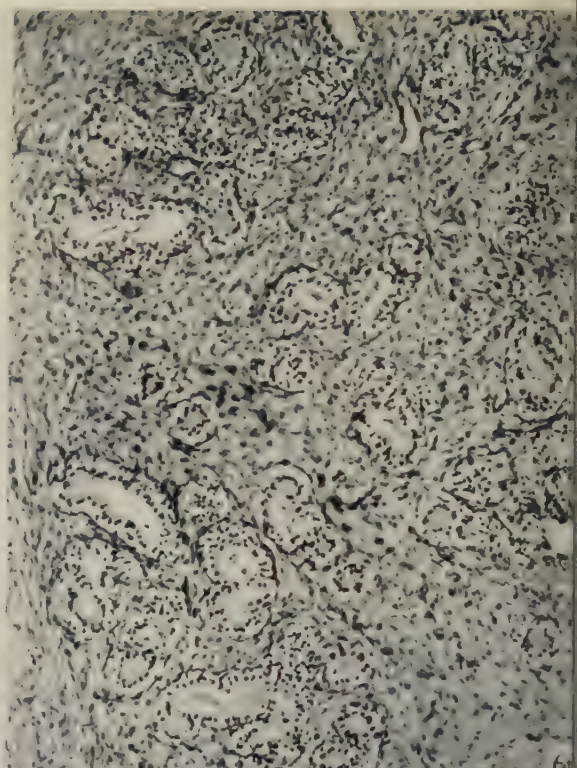
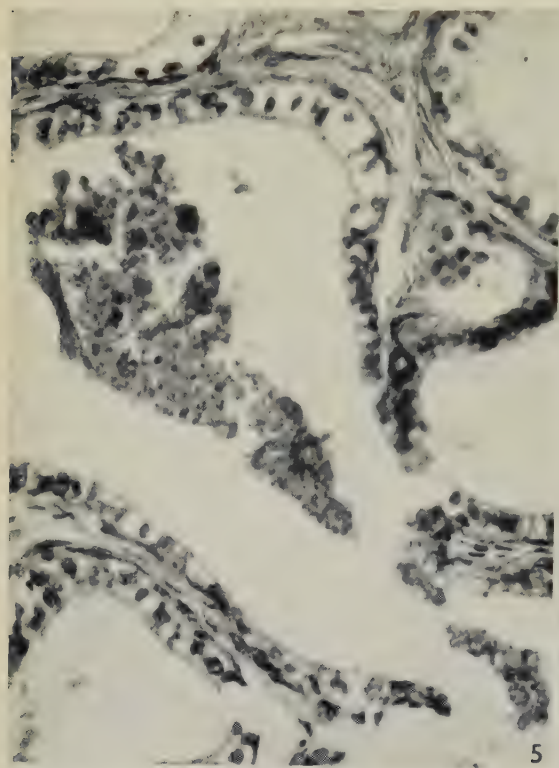
## PLATE 1

- Fig. 1. Seminal vesicle—normal ram. Haematoxylin and eosin.  $\times 95$ .
- Fig. 2. Seminal vesicle—normal ram, showing cytoplasmic globules. Haematoxylin and eosin.  $\times 400$ .
- Fig. 3. Seminal vesicle—normal ram, PAS.  $\times 400$ .
- Fig. 4. Seminal vesicle—normal ram, PAS after treatment with diastase.  $\times 400$ .









AITKEN—A HISTOCHEMICAL STUDY OF THE SEMINAL VESICLE OF THE SHEEP



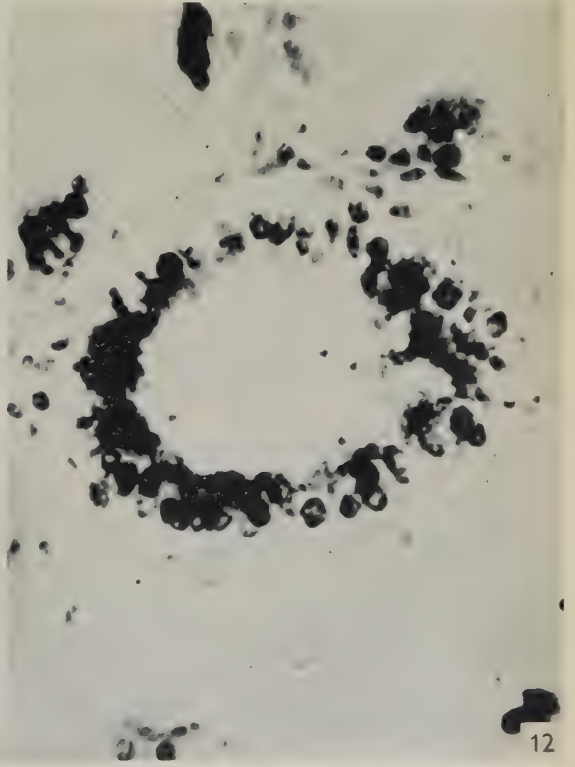
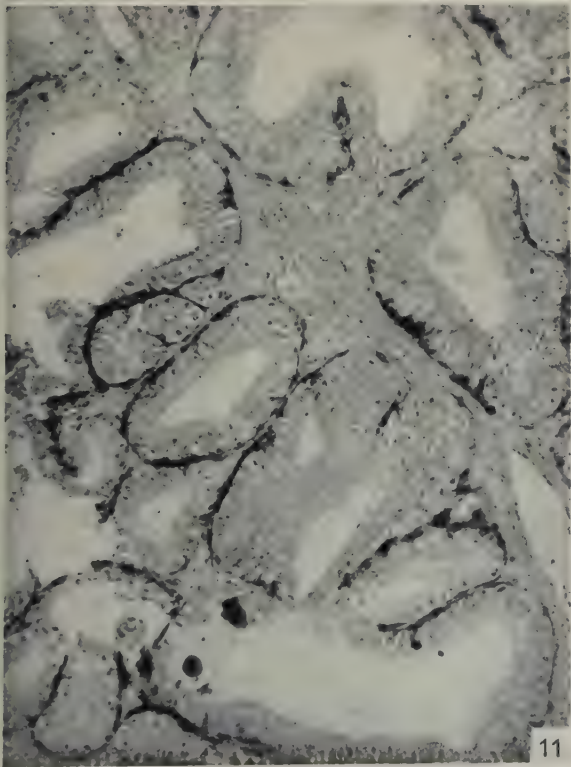
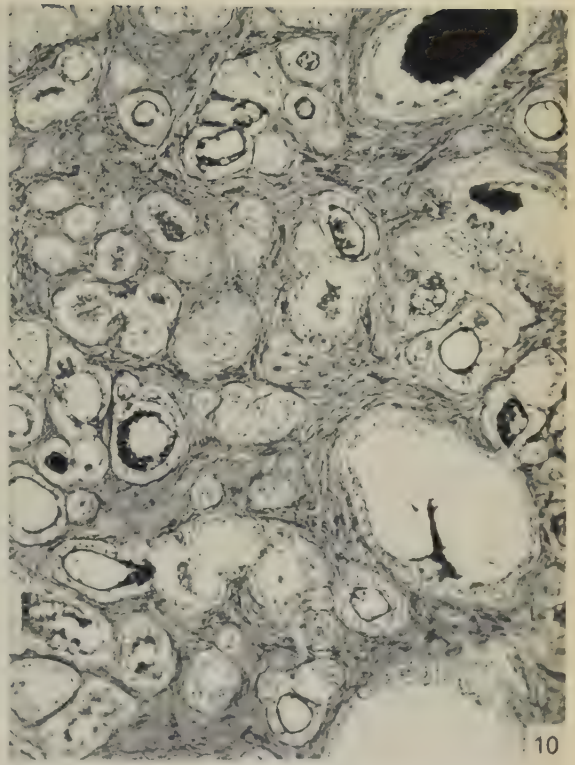
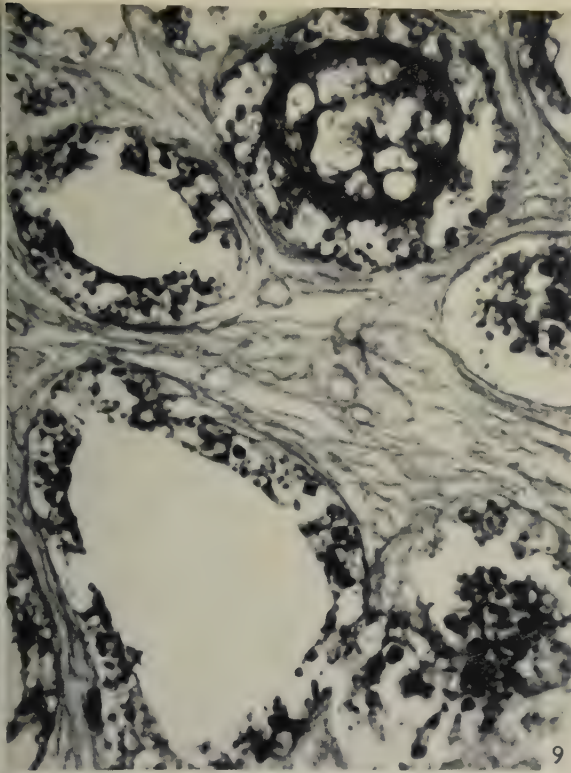






PLATE 2

- Fig. 5. Seminal vesicle—normal ram. Methyl green-pyronin.  $\times 400$ .  
Fig. 6. Seminal vesicle—castrate sheep. Haematoxylin and eosin.  $\times 95$ .  
Fig. 7. Seminal vesicle—castrate sheep, showing stratification of epithelium and regularity of free surface of epithelial cells. Haematoxylin and eosin.  $\times 420$ .  
Fig. 8. Seminal vesicle—castrate sheep. Sudan black B.  $\times 400$ .

PLATE 3

- Fig. 9. Seminal vesicle—castrate sheep, PAS, showing cytoplasmic glycogen.  $\times 420$ .  
Fig. 10. Seminal vesicle—castrate sheep, PAS, after treatment with saliva; stained material in lumen only.  $\times 95$ .  
Fig. 11. Seminal vesicle—normal ram, alkaline phosphatase (non-specific); 2 hr. incubation; no counterstain.  $\times 95$ .  
Fig. 12. Seminal vesicle—normal ram, acid phosphatase, 4 hr. incubation; no counterstain.  $\times 400$ .

# HISTO- AND BIOCHEMICAL STUDIES OF ALKALINE PHOSPHATASE IN GUINEA-PIGS WITH EXPERIMENTALLY PRODUCED OBSTRUCTIVE JAUNDICE

BY F. JACOBY AND B. F. MARTIN\*

*Department of Anatomy, University College, Cardiff*

In a previous paper (Jacoby & Martin, 1951) which dealt with a comparison between the alkaline phosphatase (A.P.) content of gall-bladder bile and the histochemically detectable A.P. in the biliary tract, it was found that in the bile of guinea-pigs A.P. was either absent altogether or present only in traces, thus accounting for the well-known A.P. negativity of the bile capillary picture of this species. From this finding two problems arose: (a) What kind of histochemical changes with regard to A.P. would occur in the liver of guinea-pigs in which the common bile duct (C.B.D.) had been ligated; in particular, was it perhaps possible to obtain, even in this species, a positive A.P. reaction in the bile capillaries by causing bile congestion in the liver? (b) What light, if any, could be thrown by the outcome of such experiments on the still vexed problem of the rise in serum A.P., which in man and some experimental animals follows obstruction of the common bile duct?

With regard to the first question it has been shown by Wachstein & Zak (1946, 1950), and by Hard & Hawkins (1950), that in species with a normally positive A.P. picture of bile capillaries, such as dog and rabbit, the intensity of this reaction after bile duct ligation is enormously increased. In the rat, in which the liver parenchyma is more often A.P. negative than not, Jacoby (1947) was sometimes successful in visualizing the bile capillaries by means of the A.P. reaction after bile duct ligation (Pl. 1, fig. 1); but the results were inconsistent, and the conditions under which a wide-spread positive result occurs are not yet known.

It can already be stated here that ligation of the C.B.D. in the guinea-pig never resulted in a general positive A.P. picture of the bile capillary system, regardless of whether the gall-bladder was left *in situ* or not.

Therefore, the remaining points, which deal with the histochemical liver A.P. and with bile-, and serum A.P. in experimental obstructive jaundice, form the major issue of this paper. In 1933, Roberts reported that obstructive jaundice in man is accompanied by a marked rise in serum A.P. His findings have been amply confirmed by many investigators, but his contention that obstructive forms of jaundice are marked off, in this respect, from other types (e.g. toxic hepatitis) has not been universally corroborated (see Morris & Peden, 1937). In spite of much experimental work on the subject, the source of the increased serum A.P. is still unknown, and the solution of the problem is hampered by lack of certainty as to the origin of both serum and bile A.P. For that of the serum, the osseous origin proposed by Armstrong & Banting (1935), has been fairly generally accepted.

With regard to the A.P. of bile, present in appreciable amounts in some species

\* Present address: Department of Anatomy, The University, Sheffield.

(e.g. man, dog, rabbit), it is not yet settled whether it is to be regarded purely as an excretion from the serum or produced by the liver, or derived from both sources. Consequently, several theories exist to explain the rise in serum A.P. in obstructive jaundice. The most widely held view is that it is due to simple retention in the serum of A.P. that would normally be excreted via the bile, whether its source be from the serum, the liver, or both. This is commonly known as the 'retention theory'. Other theories which have been put forward will be dealt with in the Discussion.

Experimental work on animals designed to cast light on this problem has included studies on the changes in the level of serum A.P. and on the histochemical changes in the liver following ligation of the C.B.D. In the dog (Bodansky & Jaffe, 1934; Armstrong & King, 1934; Freeman, Chen & Ivy, 1938; Kritzler & Beaubien, 1949; Wachstein & Zak, 1950) and rabbit (Jalling, Laursen & Volqvartz, 1945; Hard & Hawkins, 1950; Wachstein & Zak, 1950) a marked rise in serum A.P. occurs following the operation, and histochemically, as already mentioned, there is a definite intensification of the A.P. reaction (especially of bile capillaries) in the liver. In the cat no such rise was found (Cantarow, Stewart & McCool, 1936; Flood, Gutman & Gutman, 1937) or only a very moderate one of doubtful significance (Dalgaard, 1948). The reason given is that the cat excretes the enzyme in the urine, and it is of interest to mention that the kidney glomeruli are heavily A.P. positive in this species. For the rat, a rise was only found if and when the animals had been starved for at least 2 days prior to operation (Dalgaard, 1947).

The guinea-pig does not seem to have been investigated from this point of view; yet, having no A.P. output via the bile, is of obvious interest in connexion with the problem of the raised serum A.P. in obstructive jaundice. Therefore histochemical studies on the liver and gall-bladder were combined with biochemical determinations of serum and bile A.P. in guinea-pigs, in which the C.B.D. had been ligated.

#### MATERIAL AND METHODS

Twenty-eight guinea-pigs (not counting control animals) of varying ages and both sexes were used for the experimental part of this work. On the first eight of these, cholecystectomy was performed in addition to the ligation of the C.B.D. This was done in order to remove a potentially large bile reservoir and thus to increase bile retention within the liver itself. But this operation was not well tolerated by the animals; only one animal survived for 6 days. Cholecystectomy was therefore abandoned; it is actually not essential for producing a highly dilated biliary system, as the rate of bile flow in guinea-pigs is very high (see Cameron & Oakley, 1932). Leaving the gall-bladder *in situ* had an additional advantage: large quantities of bile (up to 10 ml.) could thus easily be obtained at post-mortem for biochemical A.P. determination.

Of the twenty animals in which only the C.B.D. was ligated, six died at varying times after operation; the other fourteen were sacrificed 4–6 days after operation.

Prior to operation, the animals were fasted for about a day. The operation of ligation of the C.B.D. was carried out aseptically under ether anaesthesia. The duct was exposed by a mid-line incision and, after careful isolation, ligated with cotton-thread fairly close to its entrance into the duodenum. Handling of the liver, which is very



friable, was avoided as much as possible. After ligation of the duct, the abdominal wall was closed with thread sutures.

From fourteen guinea-pigs, operated in this way, estimations of serum A.P. are available. In the first three of these (nos. 39, 40, 41) serum A.P. values were obtained only at the termination of the experiment (5 days *post operationem*) and compared with those of four normal control animals from the same stock and of similar age. Such comparison is, however, open to objections: there is a fairly wide variation of serum A.P. values amongst normal animals. Age is a particularly important factor; young animals show higher values than older ones. Daily fluctuations that may occur *post operationem* escape detection, and above all, pre- and post-operative values of serum A.P. are obviously best compared within the same animal. Therefore, from the other eleven animals blood for serum A.P. was obtained by cardiac puncture—a method not devoid of risk—first before operation and then at intervals following the operation. The last sample was taken from the neck vessels when the animals were sacrificed by decapitation under ether anaesthesia.

The A.P. content of the serum, and also of the centrifuged bile obtained at post-mortem, was determined by King's (1947) method, using a Klett colorimeter, the results being expressed in King-Armstrong (K.A.) units.

At autopsy, the gall-bladder was found grossly distended, sometimes so much so that the mucosa was herniating through the muscle coat. In two instances (nos. 21, 56) the gall-bladder had just burst; in no. 56 this occurred at the moment of opening the abdominal cavity. The viscera showed a jaundiced tinge, and in some cases there was some bile-stained free fluid in the peritoneal cavity. The ligature was always found firm and in place distal to the much distended extrahepatic bile ducts. The liver appeared somewhat enlarged and pale and, in most cases, showed a varying number of yellowish areas of necrosis both on free and cut surfaces. In two animals (nos. 13, 57) an entire lobe had undergone almost complete necrosis.

Specimens were taken from the liver and gall-bladder, fixed in 80% alcohol overnight, and embedded in paraffin at 56° C. in the usual way. Sections were cut in series at a standard thickness of 7  $\mu$  and treated histochemically to show the presence of A.P. by Kabat and Furth's modification (1941) of Gomori's (1939) technique. The sections were incubated for from  $\frac{1}{2}$  to 18 hr., with a control section at the 18 hr. period. In addition, at least one of the sections in the series was stained with haematoxylin and chromotrop for histological study.

#### EXPERIMENTAL RESULTS

(a) *Histological*: The changes that occur in the liver of the guinea-pig following bile duct ligation are well known since the early work of Charcot & Gaubault (1876). The present observations, therefore, need only a brief summary. Most sections contained at least a few, and sometimes many, areas of necrosis, irregularly distributed. They were of two kinds, the one composed of somewhat shrunken cells, staining rather intensely with chromotrop and often having pyknotic nuclei; the other consisting of pale, swollen, vacuolated cells, many of which showed karyolysis. In size the necroses varied from a small group of liver cells to that of a whole lobule. The intrahepatic bile ducts, especially after longer survival, were found grossly

dilated and tortuous, and many were filled with yellowish green bile. Numerous new bile ducts had obviously been formed, and there was much proliferative activity in the periportal connective tissue, often amounting to proper cirrhotic changes with isolation of groups of liver cells from adjacent lobules. The bile capillary channels were, particularly at the periphery of lobules, often found distended with somewhat flattened liver cells around them forming an alveolar-like arrangement. Mitotic figures were seen in bile duct epithelium and also in liver cells. There was a varying degree of leucocytosis in vessels, sinusoids and in many of the necrotic foci, which ranged from a moderate to a very excessive one. In one case (no. 16), there was an ascending cholangitis with liver abscesses.

Pl. 1, fig. 2, shows a region in which a variety of these changes can be seen.

Sections of the gall-bladders showed in some cases slight inflammatory processes in the serous membrane with leucocytic infiltration. In one animal (no. 57) there was a severe cholecystitis. Generally, the epithelium was intact over most of the circumference. It was sometimes found flattened in cases of early death, but after longer survival it was columnar with the cells closely set. In some specimens an unusually large number of mitoses were seen. This observation has since formed the subject of a special study (Jacoby, 1953).

(b) *Histochemical*: The polymorphonuclear leucocytes of the guinea-pig are always heavily A.P. positive. The normal liver of this species does not give any A.P. reaction apart from such leucocytes and occasional littoral cells of the sinusoids.

In the experimental livers, any abnormal degree of leucocytosis was, therefore, always strikingly displayed. For instance, in Pl. 1, fig. 3, practically all the black spots are due to leucocytes.

No A.P. reaction was seen, even after the longest incubation period, in bile capillaries, bile duct epithelium or in healthy liver cells.

The main places which showed an abnormal A.P. reaction were some of the necrotic areas; of these it is the 'dark' ones which usually gave a positive reaction, whilst the 'pale' ones were negative (Pl. 1, fig. 3). The positive reaction in very early foci was due entirely to the infiltration with polymorphonuclear leucocytes (Pl. 1, fig. 4*b*). In more advanced 'dark' foci, apart from leucocytes, a reaction was also given by the necrobiotic liver cells themselves, both the shrunken nuclei and the cytoplasm 'staining' darkly (Pl. 1, figs. 4*a*, 5). Occasionally, in and near such foci the sinusoidal walls were outlined in black (Pl. 1, fig. 5). The reaction in these foci was sometimes so intense, that an exact analysis and interpretation was not possible. Nuclei of healthy liver cells situated near such heavily reacting foci would often show a pseudo-reaction due to diffusion of reaction products (Martin & Jacoby, 1949; Gomori, 1950; Novikoff, 1951).

Bile, contained in many of the distended ducts, was nearly always yellow in colour i.e. non-reacting, and homogeneous in appearance, though sometimes a granular black precipitate was present as well, either a few granules (Pl. 1, fig. 7) or even a heavier precipitate, which in most cases was equally dark in the control sections (Pl. 1, fig. 6) and blue in haematoxylin preparations. This was therefore considered to be most likely some preformed calcium salt. In one liver (no. 50) a proper laminated stone was found in a dilated bile duct (Pl. 1, fig. 8).

In a few instances (nos. 16, 40, 55, 57) polymorphs were present within the lumina

of some bile ducts (Pl. 1, fig. 9), and it may well be that leucocytes in bile, by their breakdown, account for the occasional small amounts of A.P. found in centrifuged guinea-pig bile (see below), and perhaps also for the very rare observation of some A.P. positive granular material in bile ducts, which was not brown or black in control sections. It is also not impossible that necrobiotic liver cells may contribute traces of A.P. to the bile.

The gall-bladders of most of the operated animals, apart from leucocytic infiltration present in some cases, did not show any histochemical features differing from those of the normal animal. Only where wall necrosis was present due to established or imminent perforation was there a heavy diffuse A.P. reaction in this area. The lining epithelium was always negative for A.P., and so was contained bile when present. For a black granular precipitate in the lumen the same comments hold as given above.

(c) *Biochemical*: The A.P. values for bile, drained from the gall-bladder at the termination of the experiments, and for serum obtained before and at varying intervals after the operation, or only at death, are given in Table 1.

Table 1

Guinea-pig no.	Serum alkaline phosphatase (κ.A. units/100 ml.)							Bile A.P. at death κ.A. units/100 ml.
	Before operation	Days after operation						
		1	2	3	4	5	6	
20	—	—	—	—	—	—	*	0.4
22	—	—	—	—	—	—	*	0.0
51	—	†	—	—	—	—	—	0.9
52	—	—	—	†	—	—	—	3.0
53	—	—	—	—	†	—	—	4.0
39	—	—	—	—	—	13.9	—	0.3
40	—	—	—	—	—	11.8	—	Clot in gall-bladder
41	—	—	—	—	—	9.5	—	
50	5	—	8*	—	—	—	—	
69	5	—	8	10†	—	—	—	†
60	6	—	9†	—	—	—	—	0.0
49	6	—	—	—	18*	—	—	1.0
68	6	—	—	—	5	5	—	0.0
70	6	—	—	6	—	8	—	0.0
66	7	—	—	—	10	10	—	0.0
55	11	—	—	—	8	11	—	7.0
57	16	—	—	—	—	27	—	2.0
56	19	—	—	12	13	20	—	Gall-bladder burst
58	22	—	—	13	13	13	—	
Serum A.P.								
32	Controls to 39-41	13						
33		12						
34		12.5						
35		13.9						

For the first five animals A.P. values for bile only exist.

\* Animal killed.

† Animal died.

‡ No determination.

All the other animals were sacrificed on the 5th day.

Gall-bladder *bile* was available from sixteen of the animals studied. In six of these there was no A.P. detectable in the bile; in four there were only traces of A.P. such as have previously been reported by us (1951) for the bile of normal guinea-pigs; and in



six slightly higher values were found. In most of these latter animals signs of inflammation were present in the gall-bladder; these slightly raised A.P. values are therefore easily accounted for by breakdown of A.P. positive polymorphs, and possibly also due to admixture of inflammatory exudate. It is certainly significant that polymorphs were actually seen in intrahepatic bile ducts of some of these animals (nos. 55, 57).

*Serum* A.P. values are presented for fourteen animals. As explained on p. 442, the figures for animals nos. 39–41, when blood was obtained at death only, should be compared with the figures of four control animals (nos. 32–35). It will be seen that there is no significant difference between the two sets, the average serum A.P. value of the operated animals being 11·7 K.A. units and that of the controls 12·9 K.A. units. The figures of the remaining eleven guinea-pigs, for which repeated serum A.P. values exist, also show clearly that there is no general rise in the serum A.P. level following the operation. In seven animals it remained fairly constant; in one it showed even a slight fall; only in three animals are the values obtained at death definitely raised above the pre-operative level. These need some comments. Animal no. 69 was a very sick animal and showed a subphrenic abscess at post-mortem. In animal no. 49, numerous necroses were present in the liver, many of the 'dark' A.P. positive variety, and in animal no. 57 there was even a whole liver lobe necrotic, and also a severe cholecystitis with wall necrosis. In addition, this animal was in extremis when sacrificed, the blood taking was difficult and technically not free from objection. There were then some major complications in these three animals; and it seems fair to say that in uncomplicated cases of experimental obstructive jaundice in the guinea-pig there is—at least during the first 5 days after operation—no rise in serum A.P.

#### DISCUSSION

The histological changes seen in the livers of these guinea-pigs confirm the observations of previous workers. Focal necroses following bile duct ligation are a common feature in guinea-pigs, rats and rabbits. They hardly ever occur in dogs, even after prolonged biliary obstruction.

The histochemical A.P. picture given by the necrotic areas was similar to that reported for the rabbit by Wachstein & Zak (1950). The 'pale' areas were devoid of an A.P. reaction, whilst the 'dark' ones were often, at least partially, positive. It appears that in necrobiotic liver cells some A.P. becomes 'unmasked' and a cytoplasmic reaction is histochemically obtained; this also occurs after liver poisoning as observed by Kritzler & Beaubien (1949). Often portions of the liver sinusoids are then outlined in black. Apart from such areas and leucocytes, there was no A.P. reaction in the guinea-pig liver following the operation.

In two respects then, the guinea-pig stands out amongst other species investigated; it does not give a positive bile capillary reaction, even after ligation of the C.B.D., nor does it show, in uncomplicated cases, a rise in serum A.P.; whilst in species such as man (Sherlock & Walshe, 1947; Kritzler & Beaubien, 1949), dog (Wachstein & Zak, 1950) and rabbit (Hard & Hawkins, 1950; Wachstein & Zak, 1950) there is in biliary obstruction a parallelism between a generally increased A.P. reaction in the liver (hepatic cells, bile capillaries, sinusoids) and a rise in serum A.P. There is no

evidence that organs, other than the liver, show an increased A.P. content in obstructive jaundice (Hoffmeyer, Jalling & Schönheyder, 1946); but before attributing to the liver the major role with regard to the rise in serum A.P., there are other factors to be considered.

It has been suggested that there is a serum-A.P.-activating substance in jaundiced blood (Thannhauser, Reichel, Grattan & Maddock 1938, and others). The evidence for this has been conflicting, and the present results on guinea-pigs, which were definitely jaundiced, would contradict this theory. They also negate the idea that jaundiced blood contains a substance capable of stimulating site(s) of serum A.P. production; or that 'binding' of serum A.P. occurs to some substance in jaundiced blood, thereby preventing its elimination.

Next, it is necessary to consider briefly the source of serum A.P. in the normal animal. Evidence is strongly in favour of an osseous origin. Certain bone diseases such as osteogenic sarcoma and Paget's disease are accompanied by a rise, and others such as scurvy by a fall in serum A.P. level. Also it has been shown experimentally by Gould & Schwachman (1942) that in scorbutic guinea-pigs there was not only a fall in serum A.P., but also a decrease in the biochemically determined A.P. activity of bony tissue, whilst the A.P. activity of other organs remained unchanged. In some cases the serum A.P. of these animals approached even zero. Armstrong & Banting (1935) found that extirpation of organs other than bones, and also rich in A.P. did not lead to a fall in serum A.P.

It seems then, that it is to the liver that attention must be directed re the raised serum A.P. in obstructive jaundice. The questions to be answered are: (a) Is the liver a regulator of the normal serum A.P. level, in which case the rise in serum A.P. following obstruction would be due to a failure of the liver to excrete the A.P. from the serum? (b) Does obstruction to bile flow cause alteration of the liver parenchyma leading to the liberation of A.P. from damaged liver cells into the blood? (c) Is the liver normally (in certain species) the actual site of production of bile A.P., neither contributing to, nor regulating serum A.P.?

(a) Apart from some acute experiments involving hepatectomy in dogs (Armstrong & Banting, 1935; Dalgaard, 1949), in which a rise in serum A.P. followed the operation within a few hours, the majority of experiments dealing with this problem have failed to furnish evidence for the liver being a regulator of serum A.P. level. Excess of serum A.P. due to bile duct obstruction in dogs which is later relieved, returns to a normal level only very slowly (Armstrong & King, 1934); similarly, a raised serum A.P. due to transfusion of blood from a bile-duct-obstructed dog is also very slow to return to normal (Freeman & Chen, 1938; Cantarow & Miller, 1948). Again, if in dogs only some of the hepatic ducts are ligated, a marked rise in serum A.P. occurs within a few days, which is proportional to the number of ducts ligated; but the return to normal takes several months (Freeman *et al.* 1938; Gutman, Hogg & Olson 1940). Removal of a portion of liver corresponding in amount to that involved by ligation of an hepatic duct does not lead to a rise in serum A.P. (Freeman *et al.* 1938). Finally, Freeman has recently (1951) shown that the rise in serum A.P. in dogs is greater following bile duct obstruction than that after hepatectomy. It is therefore doubtful, or even unlikely that the liver acts merely as a regulator of the serum A.P. level; and in the guinea-pig, which does not contain A.P. in the bile, this is

most certainly not the case. Also, the elevation of the serum A.P. in certain bone diseases does not support this idea.

(b) and (c) If then the liver itself is the source of the raised serum A.P. level in obstructive jaundice of certain species, this may be due to liver cell damage or/and to mere mechanical factors. It has already been mentioned that in experiments of this kind some species show A.P. positive liver necroses. This was also found to be the case in the present work, but only when these necroses were massive was there an indication of a slight rise in serum A.P. Though possibly occasionally contributing to such a rise, they cannot represent the major factor, as they do not occur in the dog, which shows a particularly marked elevation of the serum A.P. in biliary obstruction.

It therefore seems reasonable to conclude that the main, if not only, factor concerned is a mechanical one. It is suggested that in species which normally secrete A.P. with the bile, the enzyme is actually produced by the liver cells and that in biliary obstruction it is forced to enter the blood stream either through reversal of the secretory polarity of the liver cells or through a forced flow of bile tracking into the sinusoids, thus causing the rise in serum A.P. level. It seems that the situation in the guinea-pig (no A.P. in the bile) and the results obtained with this species after ligation of the C.B.D. (no rise in serum A.P.) lend further, though indirect, support to this concept.

#### SUMMARY

1. An investigation has been made of the effects of ligation of the common bile duct in guinea-pigs on (a) the histochemical reaction for alkaline phosphatase (A.P.) in the liver and gall-bladder, and (b) the A.P. level in gall-bladder bile and in blood serum.

2. Healthy liver cells, bile capillaries and the epithelium of bile ducts and gall-bladder remained A.P. negative as they are in normal animals. Bile, stagnant in dilated intrahepatic ducts, was also found A.P. negative. Only in early focal necroses was there some evidence of a positive A.P. reaction given by degenerating liver cells, with partial outlining of sinusoids.

3. Biochemically, bile either did not contain any A.P. at all, or only very small amounts of the enzyme, which could be accounted for by various pathological conditions.

4. In uncomplicated cases no change of the serum A.P. level occurred during the experimental period (up to 5 days).

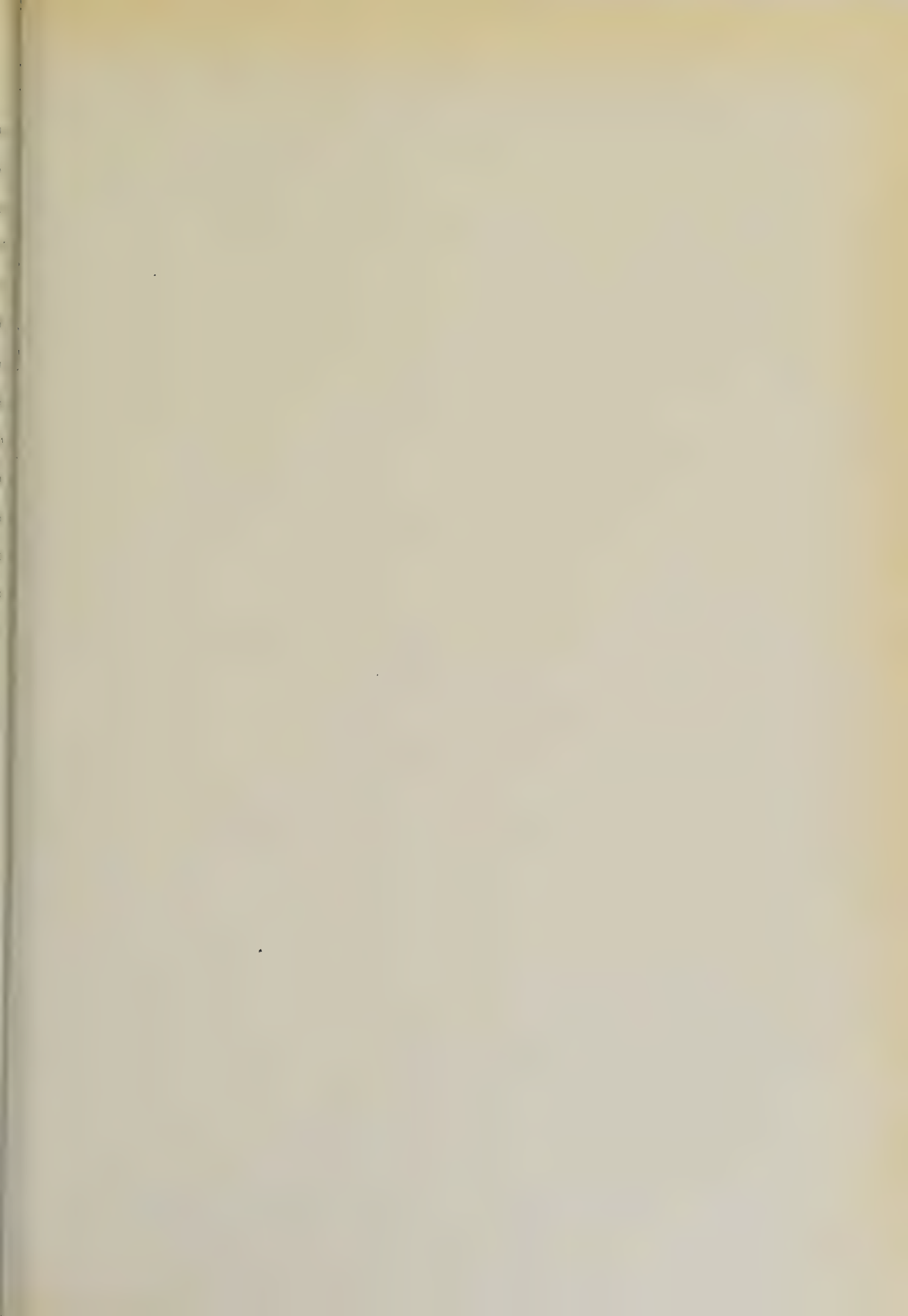
5. The results, evaluated in conjunction with other existing data on the effects of obstructive jaundice, help to exclude some of the theories concerning the rise in serum A.P., which occurs in man and some other species. They suggest that in these the source of the increased serum A.P. is the liver itself, which in these species normally produces A.P. and secretes it via the bile; in biliary obstruction this A.P. is forced to enter the blood stream.

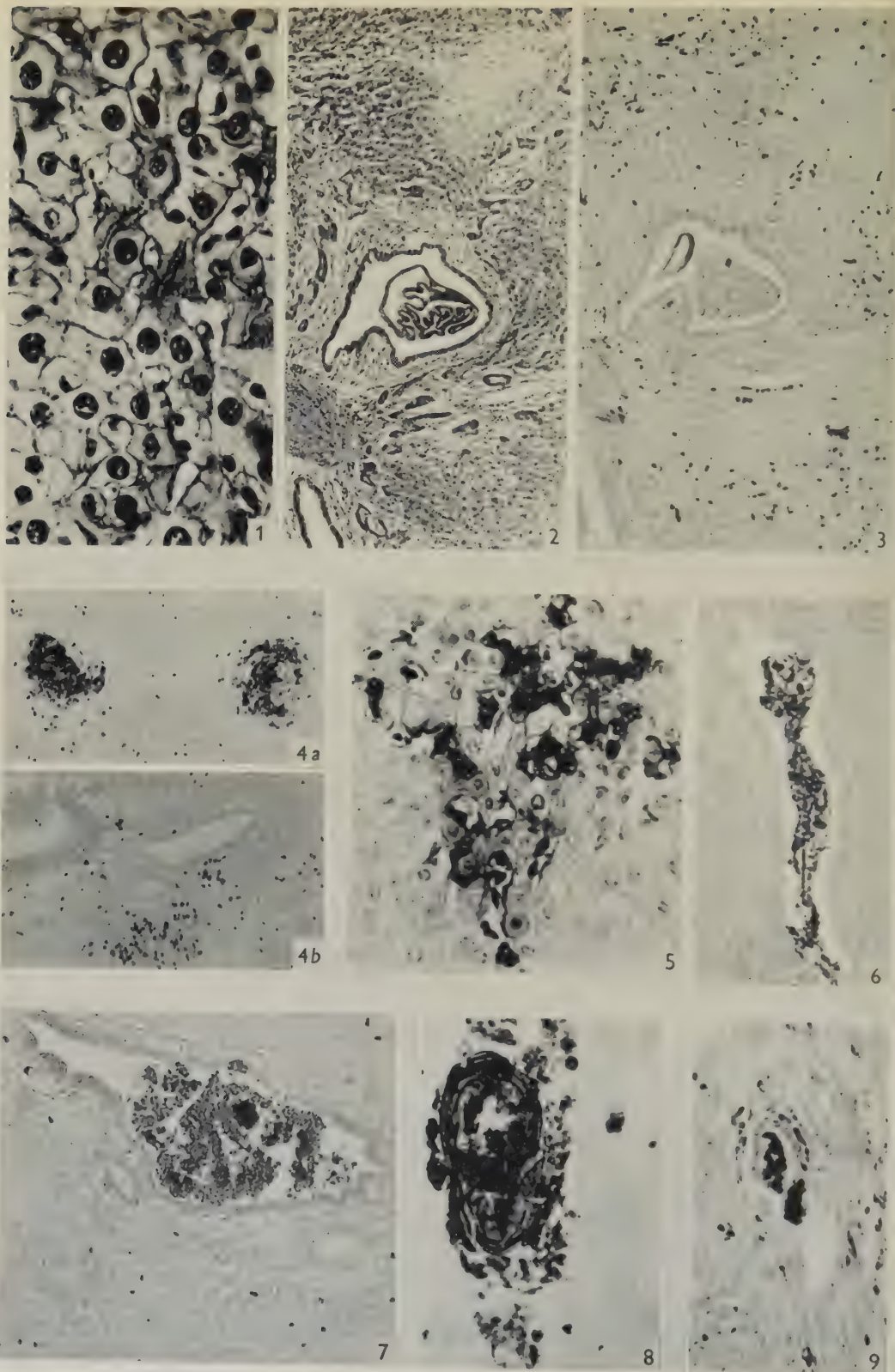
The authors wish to thank Mr L. Jones for assistance at the operations, and help with the histological preparations and microphotography.



## REFERENCES

- ARMSTRONG, A. R. & BANTING, F. G. (1935). The site of formation of the phosphatase of serum. *Canad. med. Ass. J.* **33**, 243-246.
- ARMSTRONG, A. R. & KING, E. J. (1934). Phosphatase in obstructive jaundice. *Canad. med. Ass. J.* **31**, 14-20.
- BODANSKY, A. & JAFFE, H. L. (1934). Phosphatase studies. VIII. Increase of serum phosphatase after bile duct ligation in dog. *Proc. Soc. exp. Biol., N.Y.*, **31**, 1179-1181.
- CAMERON, G. R. & OAKLEY, C. L. (1932). Ligation of the common bile duct. *J. Path. Bact.* **35**, 769-798.
- CANTAROW, A. & MILLER, L. L. (1948). Non-excretion of jaundice-serum alkaline phosphatase in bile of normal dogs. *Amer. J. Physiol.* **153**, 444-446.
- CANTAROW, A., STEWART, H. L. & MCCOOL, S. G. (1936). Serum phosphatase in cats with total bile stasis. *Proc. Soc. exp. Biol., N.Y.*, **35**, 87-89.
- CHARCOT, —. & GAUBAULT, —. (1876). Note sur les altérations du foie consécutives à la ligature canal du cholédoque. *Arch. Physiol. norm. path.* **3**, 272-299.
- DALGAARD, J. B. (1947). Serum phosphatase in rats with obstructive jaundice. *Acta physiol. scand.* **13**, 310-321.
- DALGAARD, J. B. (1948). Phosphatase in cats with obstructive jaundice. *Acta physiol. scand.* **15**, 290-303.
- DALGAARD, J. B. (1949). Serum phosphatase after hepatectomy in dogs. *Acta physiol. scand.* **16**, 308-317.
- FLOOD, C. A., GUTMAN, E. B. & GUTMAN, A. B. (1937). Serum and urine phosphatase activity in the cat after ligation of the common bile duct. *Amer. J. Physiol.* **120**, 696-702.
- FREEMAN, S. (1951). Comparison of effects of hepatectomy and of common bile duct obstruction on serum phosphatase of adult dogs. *Amer. J. Physiol.* **164**, 792-797.
- FREEMAN, S. & CHEN, Y. P. (1938). The effect of jaundiced blood upon normal dogs, with special reference to the serum phosphatase. *J. biol. Chem.* **123**, 239-246.
- FREEMAN, S., CHEN, Y. P. & IVY, A. C. (1938). On the cause of the elevation of serum phosphatase in jaundice. *J. biol. Chem.* **124**, 79-87.
- GOMORI, G. (1939). Microtechnical demonstration of phosphatase in tissue sections. *Proc. Soc. exp. Biol., N.Y.*, **42**, 23-26.
- GOMORI, G. (1950). Sources of error in enzymatic histochemistry. *J. Lab. clin. Med.* **35**, 802-809.
- GOULD, B. S. & SCHWACHMAN, H. (1942). Bone and tissue phosphatase in experimental scurvy, and studies on the source of serum phosphatase. *Amer. J. Physiol.* **135**, 485-491.
- GUTMAN, A. B., HOGG, B. M. & OLSON, K. B. (1940). Increased serum phosphatase activity without hyperbilirubinaemia after ligation of hepatic ducts in dogs. *Proc. Soc. exp. Biol., N.Y.*, **44**, 613-617.
- HARD, W. L. & HAWKINS, R. K. (1950). The rôle of the bile capillaries in the secretion of phosphatase by the rabbit liver. *Anat. Rec.* **106**, 395-412.
- HOFFMEYER, J., JALLING, O. & SCHÖNHEYDER, F. (1946). Studies on serum phosphatase activity in relation to experimental biliary obstruction in rabbits. II. *Acta physiol. scand.* **11**, 160-171.
- JACOBY, F. (1947). Use of the phosphatase reaction in a method of demonstrating bile capillaries in rats. *J. Physiol.* **106**, 33P.
- JACOBY, F. (1953). Mitotic activity in the gall bladder epithelium of the guinea-pig after ligation of the common bile duct. *J. Physiol.* **119**, 21P.
- JACOBY, F. & MARTIN, B. F. (1951). The relationship of bile alkaline phosphatase to histochemically detectable alkaline phosphatase in the biliary tract; including reference to the histology of the gall bladder epithelium. *J. Anat., Lond.*, **85**, 391-400.
- JALLING, O., LAURSEN, T. & VOLQVARTZ, K. (1945). Studies on serum phosphatase activity in relation to experimental biliary obstruction in rabbits. I. *Acta physiol. scand.* **10**, 70-77.
- KABAT, E. A. & FURTH, J. (1941). A histochemical study of the distribution of alkaline phosphatase in various normal and neoplastic tissues. *Amer. J. Path.* **17**, 303-318.
- KING, E. J. (1947). *Microanalysis in Medical Biochemistry*. London: J. & A. Churchill Ltd.
- KRITZLER, R. A. & BEAUBIEN, J. (1949). Microchemical variation of alkaline phosphatase activity of liver in obstructive and hepatocellular jaundice. *Amer. J. Path.* **25**, 1079-1104.







- MARTIN, B. F. & JACOBY, F. (1949). Diffusion phenomenon complicating the histochemical reaction for alkaline phosphatase. *J. Anat., Lond.*, **83**, 351-363.
- MORRIS, N. & PEDEN, O. D. (1937). Plasma phosphatase in disease: a review. *Quart. J. Med.* **30**, 211-230.
- NOVIKOFF, A. B. (1951). The validity of histochemical phosphatase methods on the intracellular level. *Science*, **113**, 320-325.
- ROBERTS, W. M. (1933). Blood phosphatase and the Van den Bergh reaction in the differentiation of the several types of jaundice. *Brit. med. J.* **1**, 734-738.
- SHERLOCK, S. & WALSH, V. (1947). Hepatic alkaline phosphatase: histological and microchemical studies on liver tissue in normal subjects and in liver disease and in bone disease. *J. Path. Bact.* **59**, 615-630.
- THANNHAUSER, S. J., REICHEL, M., GRATTAN, J. F. & MADDOCK, S. J. (1938). Studies on serum phosphatase activity. VI. The influence of sera with high phosphatase activity on normal sera. *J. biol. Chem.* **124**, 631-637.
- WACHSTEIN, M. & ZAK, F. G. (1946). Histochemical distribution of alkaline phosphatase in dog liver after experimental biliary obstruction. *Proc. Soc. exp. Biol., N.Y.*, **62**, 73-76.
- WACHSTEIN, M. & ZAK, F. G. (1950). Alkaline phosphatase in experimental biliary cirrhosis. *Amer. J. clin. Path.* **20**, 99-115.

# EXPLANATION OF PLATE

Fig. 2. was taken through a Wratten B Filter; all others through a Wratten G.

- Fig. 1. Rat no. 21. Liver, 6 days after ligation of the C.B.D., A.P. reaction. Many bile capillaries are clearly outlined in black, often showing a double contour in L.S.; walls of sinusoids also outlined in black.  $\times 450$ .
- Fig. 2. Guinea-pig no. 13. Liver, 5 days after ligation of C.B.D., stained with haematoxylin and chromotrop to show some of the typical histological changes: dilatation and tortuosity of bile ducts, new bile duct formation and cirrhotic changes around them; in upper right corner a 'pale' vacuolated type of necrosis.  $\times 65$ .
- Fig. 3. Liver section near to that of fig. 2. A.P. reaction (2 hr. incubation). Moderate leucocytosis. All black spots are due to A.P. positive polymorphs in the vascular spaces. The necrotic focus in upper right corner is A.P. negative, and so are the healthy liver cells and the bile duct system.  $\times 65$ .
- Fig. 4. Guinea-pig no. 50. Liver, 2 days after ligation of C.B.D., A.P. reaction (2 hr. incubation). In (a) two necrotic foci of the 'dark' type are seen, heavily A.P. positive; in (b) an early necrosis with accumulation of leucocytes. Other liver tissue A.P. negative.  $\times 65$ .
- Fig. 5. Guinea-pig no. 41. Liver, 5 days after ligation of C.B.D., A.P. reaction (2 hr. incubation). Small 'dark' necrotic focus. Positive reaction in leucocytes, littoral cells and also in some of the necrobiotic liver cells; also portions of sinusoids are outlined in black.  $\times 365$ .
- Fig. 6. Guinea-pig no. 50. Liver, 2 days after ligation of C.B.D. Control section (18 hr. incubation), to illustrate the black precipitate which was often found in bile ducts of both phosphatase and control sections, thus considered to be due to preformed calcium salts.  $\times 125$ .
- Fig. 7. Guinea-pig no. 55. Liver, 5 days after ligation of C.B.D., A.P. reaction ( $\frac{1}{2}$  hr. incubation). The bulk of the contents of the large dilated bile duct is yellow in colour in the section. Only the small granular precipitate in the right-hand corner is black. The two particularly dark areas in the bile (deep yellowish brown in the section) are of doubtful significance. Note very moderate leucocytosis and A.P. negativity of the liver tissue.  $\times 125$ .
- Fig. 8. Guinea-pig no. 50. Liver, 2 days after ligation of C.B.D., A.P. reaction ( $\frac{1}{2}$  hr. incubation), to show a laminated gall stone in a bile duct. Owing to focusing on the lamination, the tissue in the background (e.g. branch of hepatic artery) appears blurred.  $\times 265$ .
- Fig. 9. Guinea-pig no. 40. Liver, 5 days after ligation of C.B.D., A.P. reaction (2 hr. incubation). Note the accumulation of A.P.-positive leucocytes in the lumen of a bile duct.  $\times 180$ .

## THE BEHAVIOUR OF IMPLANTATION GRAFTS OF BLADDER MUCOSA

BY F. R. JOHNSON AND R. M. H. McMINN

*Department of Anatomy, University of Sheffield*

In recent years there has been increasing interest in the transplantation of tissues to repair defects of congenital or of pathological origin. The tissues which have been most commonly transplanted and which have given greatest success include skin, cornea, bone and blood vessels. Although both autogenous and homogenous transplants have been used, as far as cellular survival is concerned little success has been claimed for most homogenous transplants due to their rapid destruction by what appears to be an antigen-antibody reaction (Medawar, 1944). The problems involved in the homotransplantation of tissues have been recently reviewed by Dempster (1951), Longmire & Smith (1951) and Billingham (1952).

A study of the literature has shown that observations on the fate of grafts of bladder tissue are largely confined to autogenous material, and in these investigations interest has been centred upon the phenomenon of bone induction. Since there would appear to be considerable scope for plastic reconstruction in the urinary tract, the present experiments have been carried out to compare the behaviour of autografts and homografts of transitional epithelium when transposed to an abnormal site, and to determine the period of survival of the homograft epithelium.

### MATERIALS AND METHODS

All experiments were performed on healthy adult cats.

The animals were anaesthetized with intra-peritoneal Nembutal and the abdomen opened through a mid-line incision. The ventral wall of the bladder was incised; the dorsal wall of the bladder was invaginated from behind, and a small area of its mucous membrane was removed. This piece of tissue was divided into two; one portion which was implanted into the sheath of the left rectus abdominis muscle served as an autograft, the other which was implanted into the right rectus sheath as a homograft. In some cases the graft was placed between the rectus muscle and the anterior wall of the rectus sheath, in others between two layers of the anterior wall of the sheath. The implants were laid as flat as possible but were not stitched in position. In order to facilitate the rapid implantation of homografts, two animals were operated on simultaneously.

A total of forty-one animals was used; in four of these, autografts alone were implanted; in six, homografts alone, and in the remaining thirty-one the animal received both an autograft and a homograft.

The animals were killed 1, 2, 3, 4, 5, 6, 7, 8, 9, 12, 15, 18, 21, 28 or 48 days after operation. The implantation sites were removed and fixed in 10% formalin. After embedding, serial sections were cut at  $8\mu$ ; every 20th section was mounted and

stained with haematoxylin and eosin. In regions of particular interest, adjacent sections were mounted and stained with methyl green pyronin to facilitate the identification of plasma cells.

## RESULTS

Epithelialized cysts were always found in animals allowed to survive for more than 4 days. These cysts were formed as a result of the implantation of either autografts or homografts, and the mechanism of their formation and their fate are now described.

### *Autografts*

*Day 1.* At the end of 24 hr. the implant can be seen lying in the artificial bed created for it in the rectus sheath (Pl. 1, fig. 1). The epithelium is well preserved (Pl. 1, fig. 4) and a few cells appear to have migrated as a single layer from the margins of the graft.

*Day 2.* The migration continues and the epithelium of the graft becomes thinned with a decrease in the number of layers of cells. The epithelium now shows a marked degree of mitotic activity. This activity is found both in the cells of the graft itself (Pl. 1, fig. 5) and in the cells which have been spreading outwards from the margins of the graft (Pl. 1, fig. 2). All phases of the mitotic cycle can be observed, and while the graft epithelium in places is reduced in thickness to that of a single layer of cells, where it does remain stratified, cell division is not confined to the basal layer. At this stage it is evident that the advancing edges of the layer of spreading cells are approaching each other (Pl. 1, fig. 6). This process eventually results in the formation of a cyst which will be lined partly by the epithelium of the original graft and partly by the epithelium which has spread and grown from the margins of that graft.

*Days 3-5.* Mitotic activity in the graft epithelium and in the spreading epithelium continues, and appears to occur at the same rate as was noted at the 2nd day. Towards the end of this period, the free margins of the spreading epithelium meet and give rise to a completely epithelialized cyst (Pl. 2, fig. 8). At first the spreading epithelium lies in close apposition to that of the graft, but with the increasing accumulation of an acellular straw-coloured fluid within the cyst, the two layers of epithelia become further separated, and eventually the cyst becomes spherical in shape. The fluid in different cysts may be under differing tensions, as in some cases the epithelium is of a flattened pavement type, while in others where possibly the pressure is low the epithelium is more cuboidal or columnar.

During this period the graft epithelium regains its original depth of stratification, and the spreading cells become multi-layered. With haematoxylin and eosin staining it has been found that the cells of the spreading epithelium differ from those of the graft in that they are larger and that the cytoplasm stains less densely (Pl. 1, fig. 3). This difference in appearance facilitates the identification of the two epithelia. It is worthy of note that although the graft epithelium has undergone thinning, a process which is followed by proliferation and stratification, its cells are always normal in size and appearance.

*Days 6-12.* With the establishment of stratification, proliferative activity diminishes and at the end of this period it has fallen to the degree usually seen in transitional epithelium.



*Days 15-18.* Once the spreading epithelium has become stratified, its cells decrease in size until they approximate to those seen in normal bladder epithelium (Pl. 1, fig. 7). It then undergoes no further change. It can, however, be distinguished from graft epithelium by the fact that there is no subepithelial plexus of blood vessels. Judging by palpation of the abdominal wall in animals allowed to survive for several weeks, the cyst does not appear to increase in size after the 2nd week.

In fifteen autografts out of nineteen, bone formation took place in the tissues underlying the new epithelium. This finding in the cat supports the contention of bone induction by transitional epithelium as suggested by Huggins (1931) and subsequent workers in other species.

It was noted that cellular infiltration around autografts is minimal and that when it is present it is confined almost entirely to infiltration with polymorphonuclear cells.

#### *Homografts*

*Days 1-4.* The appearances during the first 4 days, including the early onset of mitosis on the 2nd day, are similar to those seen in the autografts.

*Days 5-12.* Following the formation of an epithelialized cyst at approximately the 5th day (Pl. 2, fig. 9) cellular infiltration occurs. This is seen in the connective tissue surrounding the cyst and in the substance of the graft itself. At first the infiltration is confined to the subepithelial tissues (Pl. 2, fig. 10), but later when it becomes more intense there is invasion of the epithelium. The invading cells in the preliminary stages of infiltration are mainly polymorphonuclear with a few scattered lymphocytes, but these rapidly give place to lymphocytes mixed with some plasma cells.

*Days 15-28.* During the 3rd or 4th week there is evidence of cellular destruction. At first the epithelial cells can be seen to become separated from each other, to lose their characteristic appearance and finally to disintegrate.

Following the destruction of the epithelium, the cavity of the cyst becomes filled by invading lymphocytes and plasma cells (Pl. 2, fig. 11). These are soon followed by numerous fibroblasts and thin-walled blood vessels. In the early stages of invasion by these latter tissues the fibroblasts are large, with easily definable cytoplasmic processes, and are arranged in a haphazard manner. Later they are more tightly packed, the cytoplasmic processes are less easily seen, and they are now arranged in a more regular fashion (Pl. 2, fig. 12). The subsequent fate of the subepithelial tissues of the graft has not been studied. As in the case of autografts, bone formation was found to occur in areas immediately underlying the new epithelium (Pl. 2, fig. 13). In the present series bone was present in twelve out of twenty-one homografts.

Although the number of experiments has been small, the fates of the grafts in animals which have received one graft of a particular kind and in animals which have received the two types of graft, i.e. both an autograft and a homograft, do not seem to differ.

#### DISCUSSION

The findings show that in the cat, as in the dog and rabbit (Huggins, 1931), and in the rat and guinea-pig (Huggins, McCarroll & Blocksom, 1936), the implantation of an autograft of transitional epithelium results in the formation of a cyst. The tendency to form a vesicle appears to be typical of several types of epithelia when implanted into abnormal sites (Bacsich & Wyburn, 1947; Medawar, 1948; Billingham & Medawar, 1950; Hopper & Mathews, 1953). In the present work it has been noted that during cyst formation two separate processes can be observed, namely spreading or migration of epithelium from the edges of the graft, and mitosis in both the graft epithelium itself and in the migrating cells.

The phenomenon of migration when there is a lack of continuity is a characteristic of epithelia in general. It would seem that transitional epithelium is particularly suited to spreading, since it is normally not firmly attached to subepithelial tissues and since in the urinary bladder it lines an organ which undergoes marked variations in its capacity. The thinning of the graft epithelium that has been observed on the 2nd and subsequent days, is most likely to be due to this migration of cells from the periphery of the graft. From the present studies it is not possible to say whether migration alone is responsible for the formation of the cyst or whether mitosis is a contributory factor. If the marked mitotic activity which has been noted from the 2nd day onwards is not a contributory factor, it would seem highly probable that it aids the process of stratification. Folding of the graft upon itself following implantation does not appear to be a significant factor in the formation of the cyst.

The cysts formed by bladder mucosa closely resemble those formed by skin and cornea when these are used as heterotopic implantation grafts (Bacsich & Wyburn, 1947; Medawar, 1948). The cysts of transitional epithelium have always been formed with the original outer surface of the epithelium directed inwards ('external encystment' of Medawar, 1948), though in a few cases there was some additional migration of cells around the graft itself.

In specimens examined 2 days after operation, mitotic activity is a striking feature. It would appear that this early incidence of mitosis is a characteristic of regenerating transitional epithelium, since it has also been shown to occur on the 2nd day during the healing of artificial lesions of bladder mucosa (McMinn & Johnson, 1955). Unfortunately, it is not possible to compare the present findings on implantation grafts of transitional epithelium with those on similar grafts of other stratified epithelia, owing to the fact that observations on the latter have not been made in the early stages following implantation (Bacsich & Wyburn, 1947; Medawar, 1948; Billingham & Medawar, 1950). However, the present observations and those of McMinn & Johnson (1955) on bladder epithelium suggest that increased mitosis in this tissue occurs at the same time, i.e. on the 2nd day, whether the epithelium is in its normal or in an abnormal site. This may well be the case in skin also, since increased mitotic activity in orthotopic skin grafts does not occur before the 4th day (Medawar, 1944), and in the healing of skin wounds increased activity is also delayed until this time (Hartwell, 1929).

Hence, if it can be assumed that the onset of mitosis in any particular epithelium

occurs at the same time in any type of graft as it does during the healing of an ulcer in that epithelium, it would seem fair to conclude that activity in implantation grafts of transitional epithelium occurs earlier than in similar grafts of skin. The same may apply to implantation grafts of cornea, since it has been shown by Arey & Covode (1943) that mitosis in regenerating corneal epithelium is suppressed until the 4th day.

The behaviour of bladder epithelium on implantation further resembles that seen during bladder ulcer healing, in that mitosis is found in the migrating cells on the 2nd day as well as in the graft epithelium. This early incidence of mitosis in the spreading epithelium is unlike that found in other stratified epithelia; e.g. in skin healing, mitosis is not seen in migrating cells until several days after they have become firmly fixed to the underlying tissue (Ivy, Grossman & Bachrach, 1952).

Throughout this work it has been noted that in the early stages the epithelium which spreads and grows from the margins of the graft can be easily distinguished from the graft epithelium itself by the fact that the cells are larger, the cytoplasm stains less densely and the nucleus has a more vesicular appearance. These appearances are similar to those which McMin & Johnson (1955) have found in the epithelium which initially covers the floor of artificial ulcers of the bladder. It also resembles the transitional epithelium seen in the early stages of normal development; this, in conjunction with the finding that later it adopts the normal appearance of transitional epithelium, supports the contention that it is newly formed.

The behaviour of the epithelium of the autografts and homografts has shown no differences, a phenomenon that is well recognized in other grafted tissues up to the time of homograft destruction. The cellular reaction which is provoked by the presence of autografts and homografts is similar up to the 5th day. Following this, the reactions differ markedly in that the reaction to homografts increases in intensity until the time of epithelial destruction in the 3rd or 4th week, while that to autografts tends to disappear. These appearances seem to be similar to those found by other workers in the study of the fate of other homogenous tissues.

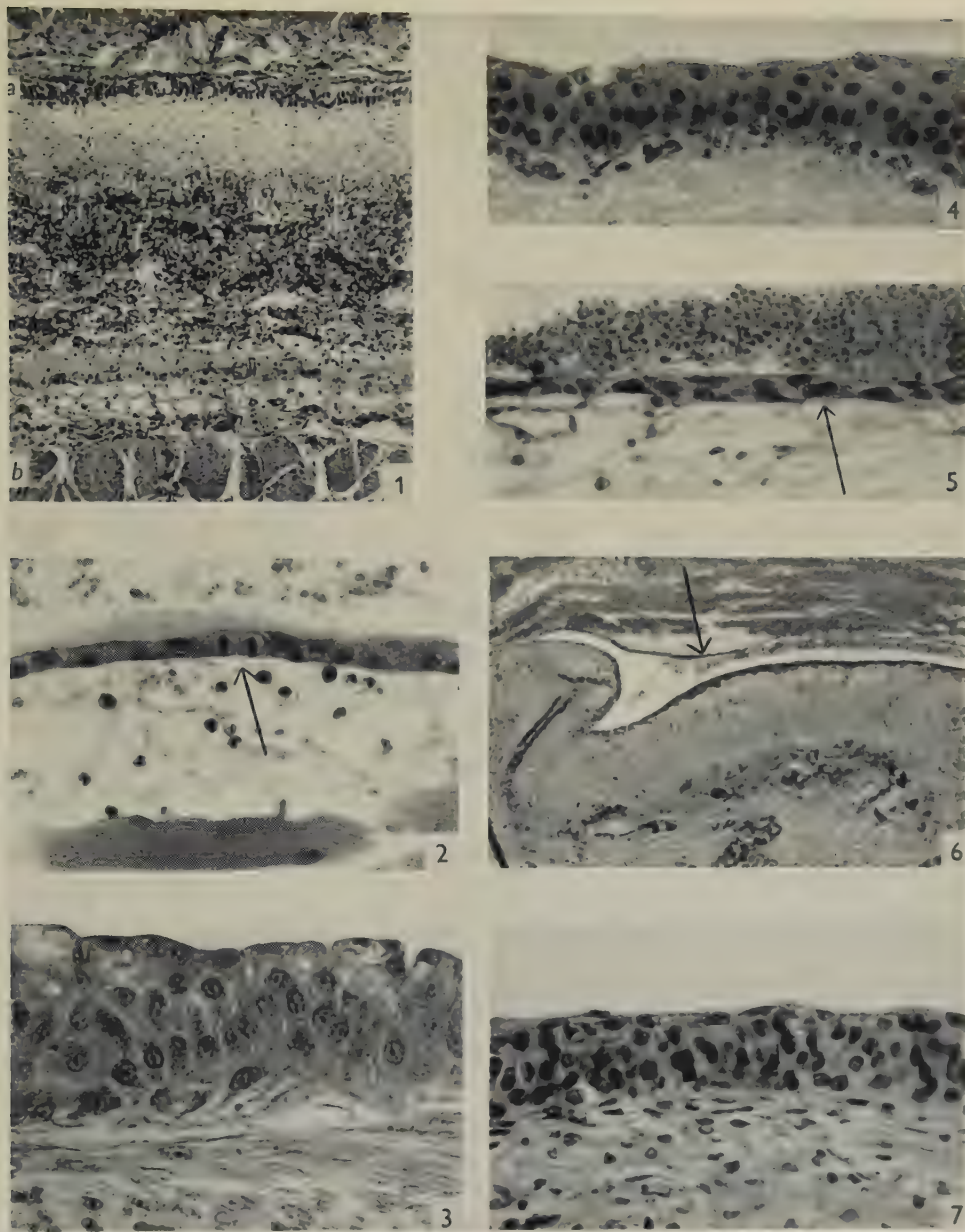
It is generally held that homograft destruction is the result of an antigen-antibody reaction, and the present findings are compatible with this concept. Judging by the rate of destruction of the homograft epithelium in these experiments it would appear that the clinical use of homografts of bladder mucosa would be no more successful than those of other epithelia. On the other hand, autografts, which show evidence of rapid growth and indefinite survival, would appear to be well suited for clinical purposes provided that they do not induce bone formation in the sub-epithelial tissues. It is interesting to note that the epithelium of homografts, like that of autografts, appears to be capable of inducing bone formation prior to the time of its destruction. This latter observation will be fully discussed elsewhere.

#### SUMMARY

The behaviour of autografts and homografts of transitional epithelium has been studied in cats following implantation into the rectus sheath.

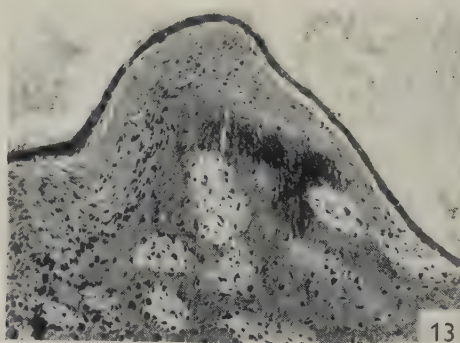
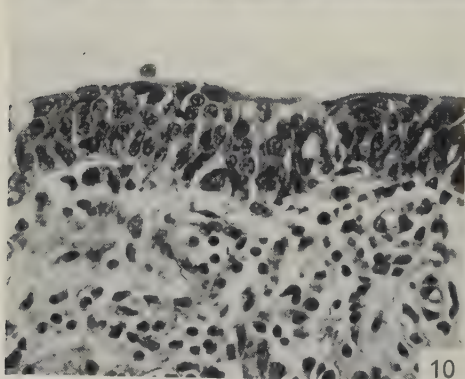
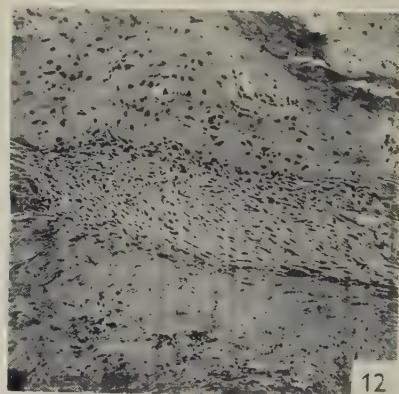
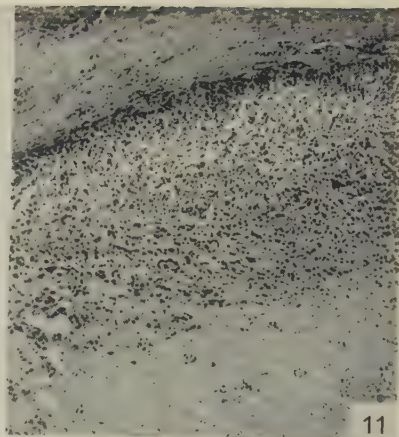
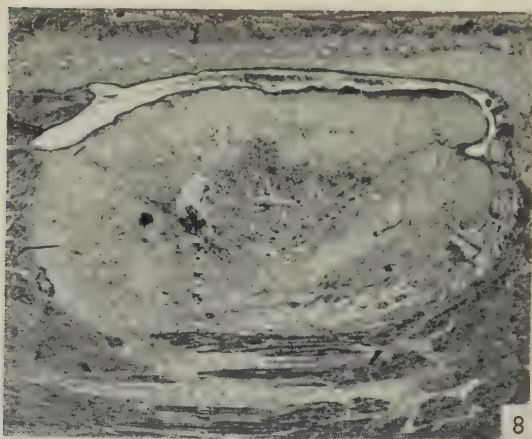
The behaviour of both implants is similar up to the 5th day. Epithelialized cysts are formed, and marked mitotic activity, commencing on the 2nd day, is seen both in the graft epithelium and in the migrating cells.





JOHNSON AND McMINN—THE BEHAVIOUR OF IMPLANTATION GRAFTS OF BLADDER MUCOSA

(Facing p. 454)



JOHNSON AND McMINN—THE BEHAVIOUR OF IMPLANTATION GRAFTS OF BLADDER MUCOSA



In autografts the epithelium persists in a healthy state; in homografts after the 5th day, there is cellular infiltration with lymphocytes and plasma cells, and epithelial destruction occurs during the 3rd or 4th week.

Homografts, like autografts, appear to be capable of inducing bone formation during the period of their survival.

The authors are indebted to Prof. Francis Davies for his encouragement and helpful criticism during the preparation of this paper. Thanks are also due to Messrs J. H. Morrill and D. A. Allen for technical assistance and to Mr J. H. Kugler for the preparation of photomicrographs. Part of the expenses of this work was defrayed by a grant from the Medical Research Fund of the University of Sheffield, and for this the authors also express their thanks.

#### REFERENCES

- AREY, L. B. & COVODE, W. M. (1943). The method of repair in epithelial wounds of the cornea. *Anat. Rec.* **86**, 75-86.
- BACSICH, P. & WYBURN, G. M. (1947). The significance of the mucoprotein content on the survival of homografts of cartilage and cornea. *Proc. roy. Soc. Edinb. B*, **62**, 321-327.
- BILLINGHAM, R. E. (1952). Homografts. *Brit. J. plast. Surg.* **5**, 1-5.
- BILLINGHAM, R. E. & MEDAWAR, P. B. (1950). A note on the specificity of the corneal epithelium. *J. Anat., Lond.*, **84**, 50-56.
- DEMPSTER, W. J. (1951). Problems involved in the homotransplantation of tissues, with particular reference to skin. *Brit. med. J.* **2**, 1041-1049.
- HARTWELL, S. W. (1929). Surgical wounds in human beings. A histologic study of healing with practical applications: I. Epithelial healing. *Arch. Surg., Chicago*, **19**, 835-847.
- HOPPER, A. F. & MATHEWS, W. W. (1953). Metaplasia and other phenomena in some intraocular transplants of adult mouse epithelia. *Anat. Rec.* **117**, 629.
- HUGGINS, C. (1931). The formation of bone under the influence of epithelium of the urinary tract. *Arch. Surg., Chicago*, **22**, 377-408.
- HUGGINS, C. B., MCCARROLL, H. R. & BLOCKSOM, B. H. (1936). Experiments on the theory of osteogenesis. The influence of local calcium deposits on ossification; the osteogenic stimulus of epithelium. *Arch. Surg., Chicago*, **32**, 915-931.
- IVY, A. C., GROSSMAN, M. I. & BACHRACH, W. H. (1952). *Peptic Ulcer*, p. 121. London: J. and A. Churchill.
- LONGMIRE, W. P. & SMITH, S. W. (1951). Homologous transplantation of tissues. A review of the literature. *Arch. Surg., Chicago*, **62**, 443-454.
- MCMINN, R. M. H. & JOHNSON, F. R. (1955). *Brit. J. Surg.* (in the Press).
- MEDAWAR, P. B. (1944). The behaviour and fate of skin autografts and skin homografts in rabbits. *J. Anat., Lond.*, **78**, 176-199.
- MEDAWAR, P. B. (1948). Immunity to homologous grafted skin. III. The fate of skin homografts transplanted to the brain, to subcutaneous tissue, and to the anterior chamber of the eye. *Brit. J. exp. Path.* **29**, 58-69.

#### EXPLANATION OF PLATES

All sections were stained with haematoxylin and eosin.

##### PLATE 1

- Fig. 1. Autograft, day 1. Part of an implant is seen lying between the rectus sheath and the rectus muscle (*b*). The epithelium of the implant is indicated at *a*.  $\times 38$ .
- Fig. 2. Autograft, day 2. A telophase (indicated by the arrow) can be seen in the epithelial cells which have spread from the margins of the graft.  $\times 340$ .
- Fig. 3. Autograft, day 5. The spreading cells are now stratified. Compare with normal graft epithelium (fig. 4) at the same magnification.  $\times 340$ .
- Fig. 4. Autograft, day 1. The epithelium preserves its normal appearance.  $\times 340$ .



- Fig. 5. Autograft, day 2. The epithelium of the graft is reduced in thickness to two layers, and a mitotic figure is indicated by the arrow.  $\times 340$ .
- Fig. 6. Autograft, day 2. The implant is lying beneath the rectus muscle and a layer of cells (indicated by the arrow) can be seen spreading from the edge of the graft.  $\times 48$ .
- Fig. 7. Autograft, day 21. The spreading cells now show a normal appearance. Compare with figs. 3 and 4.  $\times 340$ .

## PLATE 2

- Fig. 8. Autograft, day 4. A completely epithelialized cyst lying in the rectus sheath.  $\times 16$ .
- Fig. 9. Homograft, day 5. A completely epithelialized cyst lies in the sheath of the rectus abdominus muscle.  $\times 16$ .
- Fig. 10. Homograft, day 6. Round cell infiltration is seen in the subepithelial tissues. As yet the epithelium is not invaded.  $\times 340$ .
- Fig. 11. Homograft, day 18. The epithelium has been destroyed and the cyst cavity is filled with lymphocytes and plasma cells.  $\times 93$ .
- Fig. 12. Homograft, day 21. The cyst cavity is filled with organizing fibrous tissue.  $\times 80$ .
- Fig. 13. Homograft, day 15. Bony trabeculae have formed in the subepithelial tissues.  $\times 95$ .

## OSSIFICATION AND GROWTH OF THE DISTAL ULNAR EPIPHYSIS OF THE RABBIT

By P. A. RING

*Anatomy Department, Charing Cross Hospital Medical School*

Interest in the mode of growth of long bones tends to be concentrated in the changes occurring in the diaphysis and in the activities of the epiphyseal cartilage. Few observations have been made upon the development of the bony epiphyses. The histogenesis of the epiphysis has been described by Stump (1925). Haines (1933) has briefly described the relationship of the secondary centre of ossification to the cartilage canals. Payton (1933) has contributed observations upon epiphyseal growth in the madder-fed pig. Radiographs of children showing opaque striae in the epiphysis have been produced by Siegling (1941) to illustrate the mode of growth, and further illustrations are available from Gottesleben (1939) and from Boerema (1942).

In the present study, the histology of the distal ulnar epiphysis of the rabbit has been correlated with its radiological appearance and with its growth. This epiphysis appears 5 days after birth and enlarges rapidly during the first 6 weeks of life. Subsequent growth is slow, and after the 9th week of life increase in length is very slight. The ulna in the rabbit ceases growing during the 5th month of life, although bony fusion of epiphysis and diaphysis is not usually complete until the 8th month.

### METHOD

In this investigation two litters of chinchilla rabbits were used. Of the first litter, one rabbit was sacrificed at birth, and others at the ages of 1, 2, 3, 5, 7, 9, 12, 14 and 16 days. Each animal was radiographed after death using a standard technique previously described (Ring, 1955). The distal end of each ulna was fixed in Susa, and after decalcification was sectioned serially, on one side transversely, and on the other longitudinally. The histological observations upon this group were extended by the examination in longitudinal section of the normal epiphyseal cartilage of the ulna of a number of rabbits, aged from 18 days to 7 weeks, sacrificed in the course of other experiments upon bone growth.

The second litter contained four animals and was used for radiographic studies only. At the age of 18 days each animal was anaesthetized and a small lead marker was introduced into the distal epiphysis of the left ulna. Each animal was radiographed fortnightly for 4 months. The total length of the ulna, the length of the epiphysis and the distance from the lead marker to each extremity of the epiphysis was measured. Use of the right limb as a control demonstrated that the lead marker produced no local effect upon bone growth.

## OBSERVATIONS

*(a) Histological*

At birth the distal end of the ulna is completely cartilaginous. The metaphysis of the bone is highly vascular and there is a well-marked perichondrial ring. The cartilage cells beyond the metaphysis lie in well-marked columns, distal to which is a mass of cartilage cells in groups of 2, 3 or more. The distal part of the cartilaginous area shows many cartilage canals, most of them very vascular. Their arterioles arise from a group of vessels lying between radius and ulna, and after piercing the perichondrium, pass longitudinally in the cartilaginous part of the ulna.

During the first 2 days after birth the cartilaginous area enlarges more rapidly than the metaphysis advances. The cartilage canals are still vascular but sections on the 3rd day show that the canals are occupied by proliferating connective tissue and no erythrocytes can be seen. Around certain of the cartilage canals the matrix of the cartilage adopts the acidophilic stain and contrasts sharply with the basophilic ground substance of the cartilage (Pl. 1, fig. 1). The chondrocytes themselves appear unchanged.

On the 5th day after birth a secondary centre of ossification appears. It is associated with widespread changes in the chondrocytes. The nuclei of these cells degenerate, and the cytoplasm becomes vesicular. In the centre of this area of degeneration lie several blood vessels invading primary areolae, and in the walls of these areolae ossification is occurring. The secondary centre of ossification is closely associated with a blood supply which arises from a vessel of some size entering the epiphysis in a manner reminiscent of the invasion of the shaft by the artery of the primary centre of ossification (Pl. 1, fig. 3).

The spread of ossification within the cartilage is now rapid. By the 9th day of life the bony epiphysis is over 2 mm. in diameter, but is still surrounded by a cartilaginous shell, the cells of which are vesicular (Pl. 1, fig. 2). The cells of the epiphyseal cartilage adjacent to the epiphysis are arranged in short irregular columns in which the distal 3 or 4 cells are enlarged, and are separated from their fellows by a calcified ground substance. The epiphyseal bone in this region is arranged in short invading columns, many osteoblasts are visible and there is a rich vascular network. Within the epiphyseal cartilage occasional blood vessels can be seen. These blood vessels do not, however, appear in any place to become continuous with the vessels of the metaphysis, although they communicate freely with epiphyseal vessels (Pl. 1, fig. 4). The vessels run in groups of four or five within a single cartilage canal.

Sections from the older animals show that ossification of the entire breadth of the cartilage is completed by the 12th day. Subsequent increase in breadth of the epiphysis appears due to the deposition of new bone in concentric lamellae deep to the periosteum. At each end of the epiphyseal bone, however, thin columns of new bone come into contact with vesicular cartilage cells in a manner reminiscent of ossification at the metaphysis (Pl. 2, fig. 5). The arrangement of the distal cells of the epiphyseal cartilage in short columns, terminating in vacuolated cells which appear to be replaced by bone from the epiphysis, persists up to the 7th week of life; and suggests that the growth activities of the epiphyseal cartilage at this stage are bipolar



(Pl. 2, fig. 7). By the 8th week of life, however, a marked terminal plate of bone appears on the proximal aspect of the epiphysis (Pl. 2, fig. 8).

Transverse sections cut serially through the epiphyseal cartilage and metaphysis show many mitotic figures in the cartilage. These mitoses cannot be seen in longitudinal sections. They are confined to the reserve zone and their orientation suggests that division of cartilage cells occurs in the transverse axis of the bone, and contributes primarily to the increase in cartilage diameter. Sections passing transversely through the area in which the cartilage columns commence show that these columns often pass radially from the cartilage canal. In the region of the metaphysis similar sections show large numbers of osteoclasts and emphasize that the periphery of the bone in this region is undergoing absorption (Pl. 2, fig. 6).

#### *(b) Radiological*

The growth of the ulna in the first group of rabbits is indicated in Text-fig. 1. A secondary centre of ossification appears at the distal end of the ulna 5 days after birth. The epiphyseal bone increases in size rapidly and by the 16th day is 4.5 mm. long.

Growth of the ulna from the 18th day is determined from the serial radiographs of the second group of animals (Table 1). The epiphysis attains its maximum length of 7 mm. at 9 weeks although growth of the shaft of the ulna continues throughout the period of study.

The presence of the lead markers permits growth at each end of the epiphysis to be estimated (Table 2). No change occurs between the 9th and 20th week, either in the total length of the epiphysis or in the position of the marker.

### DISCUSSION

#### *(a) Histogenesis of epiphyseal bone*

In his account of the histogenesis of bone, Stump (1925) described a mesenchymal penetration of the cartilaginous epiphysis. This penetration occurs during the middle third of foetal life, and produces the canals which have been fully described by Haines (1933). The canals appear long before ossification commences in the epiphysis. It has been suggested by Haines that they are directly related to the metabolic demands of an increasingly large mass of cartilage. Hurrell (1934) agrees that these canals are unrelated to the onset of epiphyseal ossification. In the present study, the diminution of the vascularity of these canals immediately prior to the appearance of the secondary centre of ossification suggests that ischaemia may precipitate the hypertrophy and degeneration of cartilage which precedes ossification. The formation of bone, however, is associated with a large vessel passing in through the perichondrium. This mode of invasion resembles closely that of the artery of the primary centre of ossification in the shaft, and the subsequent organization of the blood supply to the epiphysis suggests that this is derived from an invasion by new vessels rather than by a reorganization of the vessels of the original cartilage canals. The blood vessels of the new epiphyseal bone freely communicate with those of the epiphyseal cartilage but do not pass into the metaphysis.



Text-fig. 1. Each figure is twice the size of the ulna determined radiologically. The figures over each bone refer to the age in days at death.

Table 1

	18 days		9 weeks		20 weeks	
	Ulna (mm.)	Ulnar epiphysis (mm.)	Ulna (mm.)	Ulnar epiphysis (mm.)	Ulna (mm.)	Ulnar epiphysis (mm.)
315	30.5	3	52.5	7	79	7
316	32	3.5	56	7	79	7
317	30	3	50	6.5	72	6.5
318	31	3.5	54	7	79	7

Table 2

	Growth of epiphysis (mm.)	Growth proximal to marker (mm.)	Growth distal to marker (mm.)
315	4	2.5	1.5
316	3.5	2.5	1
317	3.5	2.5	1
318	3.5	2	1.5

The vascularity of the cartilage at this stage is so marked that it is surprising to note that Harris (1929) and again (1933) states that normal epiphyseal cartilage is avascular, although he mentions that vessels may be seen in this area in the case of pre-existing disease leading to failure of proliferation of the epiphyseal cartilage and arrest of growth. In the rabbits studied growth was progressing normally, and no abnormality of epiphyseal cartilage could be found. Whilst it is true that in older animals the blood supply of the epiphyseal cartilage is less marked, the cartilage is rarely, if ever, avascular in the rabbit.

From the area of new bone formation at the centre of the cartilaginous mass ossification spreads centrifugally, and the extension of the secondary centre is associated with hypertrophy and degeneration of the surrounding chondrocytes. Spread of ossification permits the definition of a true epiphyseal cartilage. The epiphyseal bone in its columnar pattern, its free vascular supply and numerous osteoblasts suggests that it is actively invading the epiphyseal cartilage. The length of the adjacent cartilage columns, however, is an indication of the activity of bone growth (Becks, Kibrick, Marx & Evans, 1941) and these columns are considerably shorter than those on the metaphyseal surface of the epiphyseal cartilage. This cartilage thus presents a distinct bipolar appearance in the early weeks of life. The extent of its contributions to metaphysis and epiphysis can be judged from the relative lengths of the cartilage columns on its proximal and distal surfaces. The formation of a terminal plate of bone in the epiphysis around the 9th week of life is associated with a disappearance of the short, distal, cartilage columns.

#### *(b) Growth of the epiphysis*

The radiological studies suggest that growth of the bony epiphysis of the rabbit's ulna occurs both on its proximal and distal surfaces. Between the period of 18 days and 9 weeks the epiphyseal cartilage contributes considerably more than the articular cartilage to the growth of this bone. At the age of 9 weeks the epiphyseal bone is fully formed and no further growth in length occurs.

Payton (1933) has made observations upon the madder-fed pig at variance with these findings. The young pigs in his series were given variable periods of madder feeding succeeded by a non-madder period before death. Payton concluded, from a study of the unstained areas of bone laid down prior to death that growth occurred solely on the articular aspect of the epiphysis. His youngest animals, however, were nearly 3 months old, and since growth in the pig ceases around the age of 1 year, the early contribution of the epiphyseal cartilage to epiphyseal length would remain undetected. From calculations in which the growth rate of the epiphysis was predicted and then found to differ from the observed growth rates, Payton concluded that absorption of epiphyseal bone occurred at its junction with the epiphyseal cartilage. His method of predicting rates of bone growth, however, gives too high a value since it averages the new bone formed over a period of several weeks and applies this figure to predict subsequent growth (Payton, 1933, table VIII). Since the rate of growth of the epiphysis diminishes each week, although showing that the predicted rate exceeds the observed rate of bone growth, these calculations do not imply that absorption is occurring but merely that the methods of calculating growth



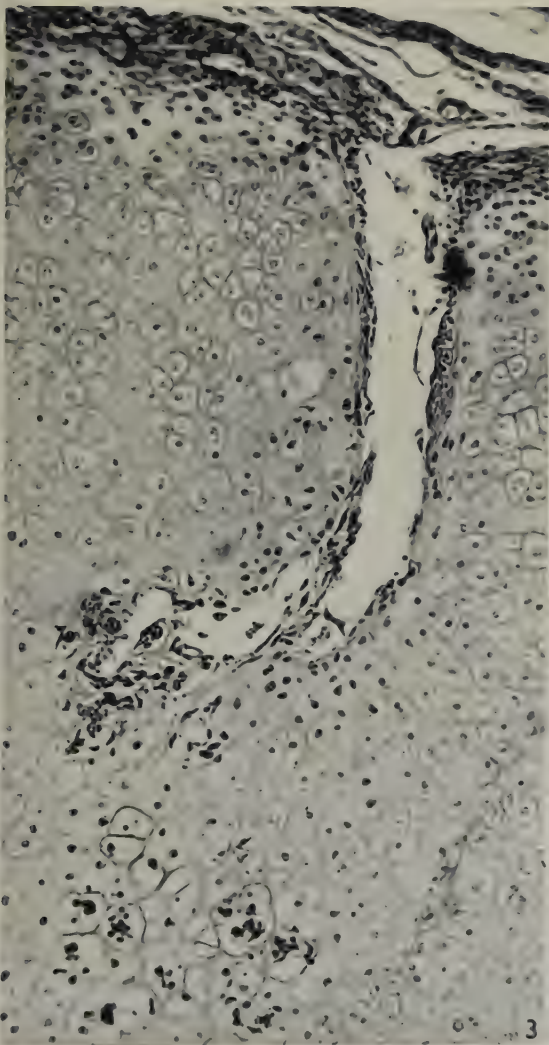
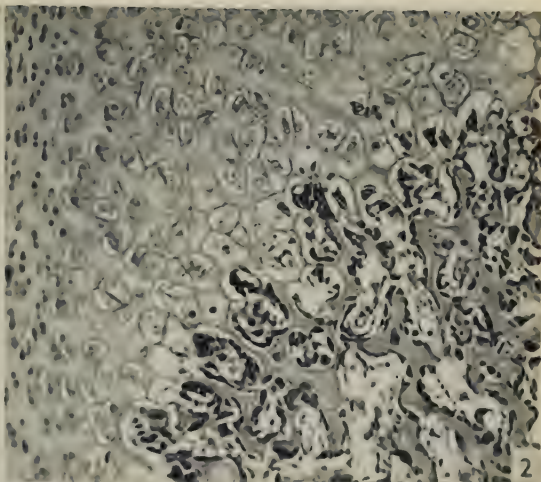
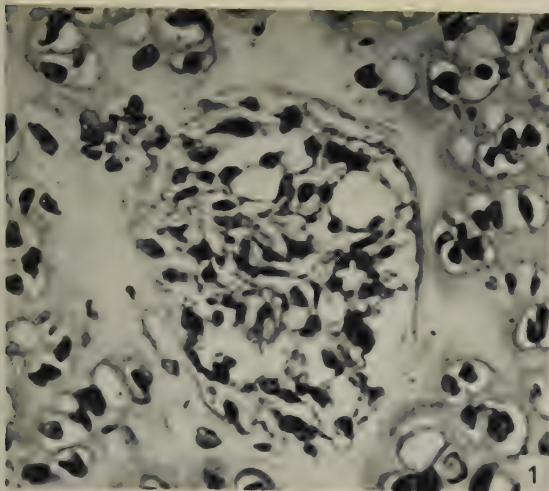
are invalid. Other criticisms may be advanced against Payton's methods of comparing different animals and indeed, in a previous paper on the growth of the diaphysis in the same pigs (Payton, 1932) he concludes: 'The fact that some of the diaphyses are shorter, whilst others are longer than their expected length may be accounted for by the circumstance that we are dealing with the bones of individual pigs with mixed inheritance, though indeed, it is notorious that individual pigs of pure breed also vary considerably in their rate of growth'.

Failure of the ulnar epiphysis of the rabbit to grow at all after the age of 9 weeks is an unexpected finding which is not applicable to other animals. Examination of the standards of Greulich & Pyle (1950) and of Todd (1937) shows that an increase in epiphyseal length occurs during the whole period of skeletal growth, although in the years prior to epiphyseal fusion growth is very slow. Siegling's (1941) radiological study of epiphyseal growth in man confirms this point. Siegling, however, suggests that the growth of the epiphysis is due solely to activity within the articular cartilage and his figures of patients with lines of growth-arrest from the ingestion of phosphorylated cod-liver oil appear to confirm this point. The illustrations of Gottesleben (1939), however, of the hip of a 3-year-old child show that increase in size of the proximal femoral epiphysis occurs originally on all surfaces of the epiphysis, and only later, as in Boerema's (1942) illustration of the hip of a 7-year-old child, is articular only. Such chance radiographic observations in man probably form the most reliable method of studying the rate and site of new bone formation.

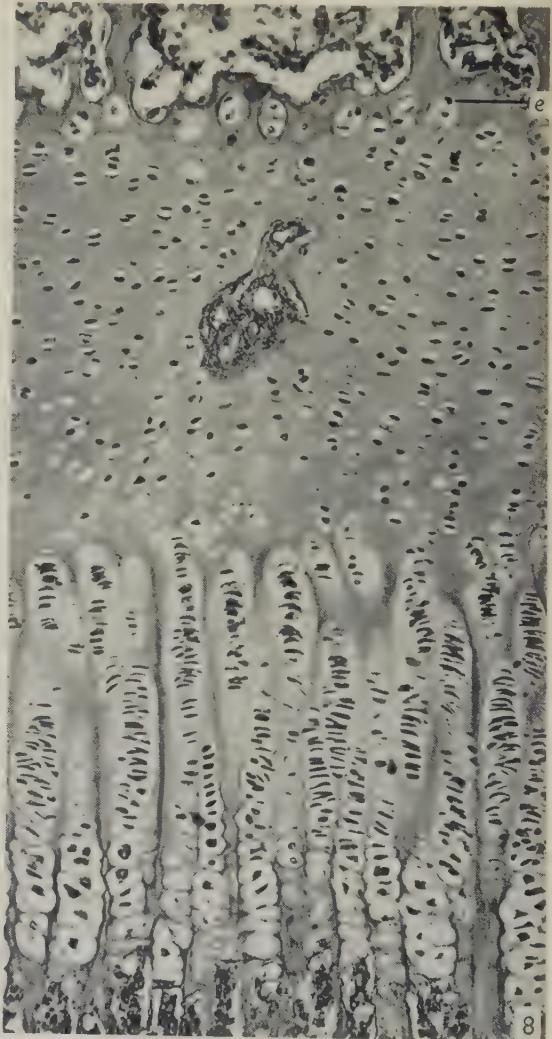
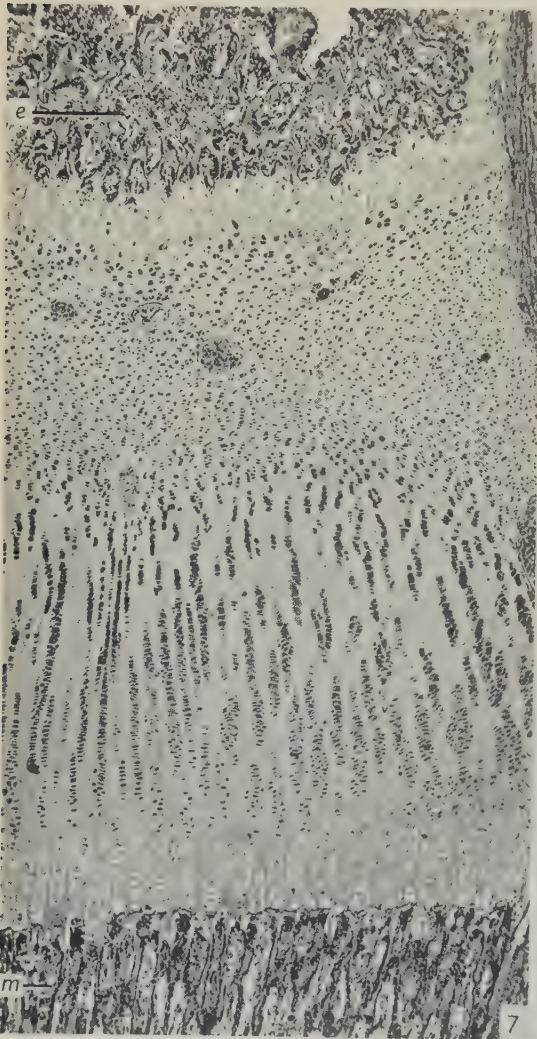
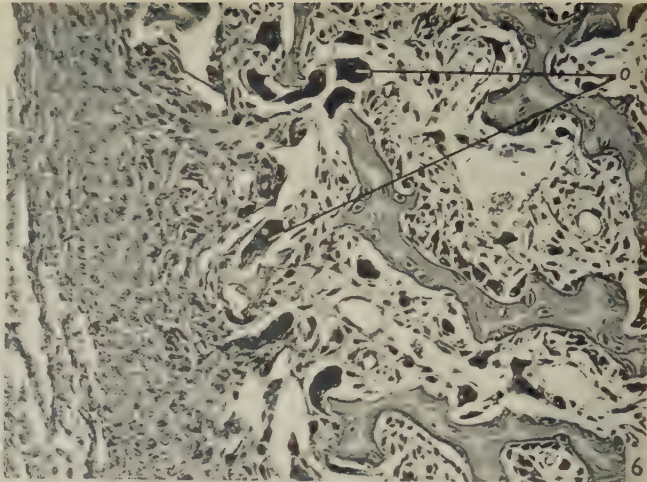
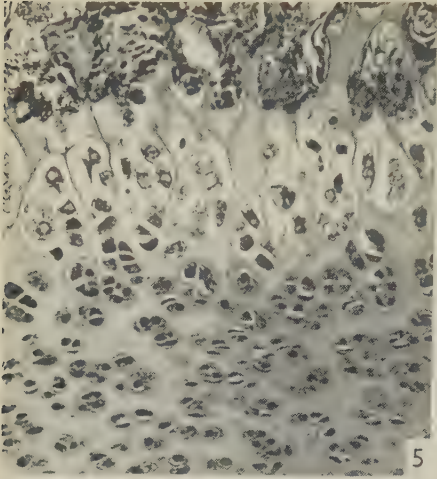
#### SUMMARY

1. The histological and radiological changes in the distal end of the ulna of the rabbit during the formation of a secondary centre of ossification are described.
2. Ossification is preceded by a widespread degeneration of cartilage cells, possibly due to a temporary ischaemia of the cartilage.
3. Invasion of the degenerating cartilage appears to occur from a large new vessel which penetrates the perichondrium.
4. Growth of the epiphysis proceeds at first by replacement both of articular and epiphyseal cartilage.
5. The formation of a terminal plate of bone is associated with cessation of growth on the diaphyseal side of the epiphysis.
6. Throughout the period of active bone growth, the epiphyseal cartilage appears well vascularized.
7. The relationship of growth in the rabbit to that in man is discussed.

I am most grateful to Prof. W. J. Hamilton for the facilities he has placed at my disposal, and for his critical reading of the manuscript. My thanks are also due to Mr R. J. McCulloch for his technical assistance, and to Miss A. Gibson for the text-figure. Mr E. V. F. Pittcock is responsible for the photomicrographs









# REFERENCES

- BECKS, H., KIBRICK, E. A., MARX, W. & EVANS, H. M. (1941). Early effect of hypophysectomy and of immediate growth hormone therapy on endochondral bone formation. *Growth*, 5, 449-456.
- BOEREMA, I. (1942). Über des Knochenwachstum. *Acta neerl. morph.* 4, 365-377.
- GOTTESLEBEN, A. (1939). In SCHINZ, H. R., BAENSCH, W. & FRIEDEL, E. *Lehrbuch der Röntgen-diagnostik*, Bd. 1, 63-65. Leipzig: Thieme.
- GREULICH, W. W. & PYLE, S. I. (1950). *Radiographic Atlas of Skeletal Development of the Hand and Wrist*. Stanford, Calif.: Stanford University Press.
- HAINES, R. W. (1933). Cartilage canals. *J. Anat., Lond.*, 68, 45-64.
- HARRIS, H. A. (1929). The vascular supply of bone with special reference to the epiphyseal cartilage. *J. Anat., Lond.*, 64, 3-4.
- HARRIS, H. A. (1933). *Bone Growth in Health and Disease*. London: Oxford University Press.
- HURRELL, D. J. (1934). The vascularization of cartilage. *J. Anat., Lond.*, 69, 47-61.
- PAYTON, C. G. (1932). The growth in length of the long bones in the madder-fed pig. *J. Anat., Lond.*, 66, 414-425.
- PAYTON, C. G. (1933). The growth of the epiphyses of the long bones in the madder-fed pig. *J. Anat., Lond.*, 67, 371-381.
- RING, P. A. (1955). The effects of partial or complete excision of the epiphyseal cartilage of the rabbit. *J. Anat., Lond.*, 89, 79-91.
- SIEGLING, J. A. (1941). Growth of the epiphysis. *J. Bone Jt. Surg.* 23, 23-36.
- STUMP, C. W. (1925). The histogenesis of bone. *J. Anat., Lond.*, 59, 136-154.
- TODD, T. W. (1937). *Atlas of Skeletal Maturation*. Part 1, Hand. London: Kimpton.

## EXPLANATION OF PLATES

### PLATE 1

- Fig. 1. A cartilage canal 3 days after birth. Between the vessels, which contain no erythrocytes, are many mesenchymal cells. The area of cartilage around the canal is eosinophilic.  $\times 400$ .
- Fig. 2. The spread of ossification from the secondary centre is centrifugal and is preceded by cartilage degeneration. Age 9 days.  $\times 150$ .
- Fig. 3. Onset of ossification in the epiphysis. A long wide channel pierces the cartilage. At its tip, new bone formation occurs. Age 5 days.  $\times 145$ .
- Fig. 4. The epiphyseal cartilage at 9 days of age. Long cartilage columns pass towards the metaphysis, short columns towards the epiphysis. A large blood vessel passes in the long axis of the bone through the cartilage.  $\times 145$ .

### PLATE 2

- Fig. 5. Four weeks after birth. The epiphyseal bone is still actively invading the epiphyseal cartilage—indicated by columns of bone with surrounding osteoblasts coming into contact with short columns of vacuolated cartilage cells.  $\times 165$ .
- Fig. 6. The periphery of the metaphysis of the ulna cut transversely. The cortex of the bone is deficient and many osteoclasts (o) are visible. Age 12 days.  $\times 160$ .
- Fig. 7. The epiphyseal cartilage 16 days after birth. The close similarity of epiphyseal (e) and metaphyseal (m) bone can be seen. The epiphyseal cartilage is vascular and is arranged in columns on each aspect, although these columns are longer and more distinct towards the metaphysis.  $\times 50$ .
- Fig. 8. The epiphyseal cartilage 8 weeks after birth. The cartilage is narrower than in the younger animal, but is still vascular. Cessation of growth on the epiphyseal side (e) is indicated by an absence of the longitudinally running columns of bone, and by the formation of a terminal plate of bone.  $\times 155$ .

## THE RENAL DUCTS OF BELLINI

By F. DURAN-JORDA

*Department of Pathology, Booth Hall Hospital, Manchester*

The main part of this research was done on neonatal kidneys containing uric acid infarcts; this physiological filling of the tubular system facilitated the follow-up of the different structures in serial sections. The precipitated material produced a casting of the whole renal medulla (Pl. 1, fig. 1), in some cases reaching up to the glomeruli. It was the existence of these deposits in the collecting tubes that made the orthodox conception of the Bellini duct opening freely into the renal pelvis appear doubtful.

A preliminary communication (Duran-Jorda, 1953) gave rise to a certain amount of interest and criticism, and though some explanations were offered (Jones & Rewell, 1954; Robson, 1954; Ross, 1954; Thung, 1954) regarding the shape of the structure described as a 'hairpin bend', no suggestions were made as to how, if the renal excretory apparatus was purely a glorified drainage system, the uric acid could precipitate and accumulate in such large amounts in the pyramids of recent-born babies, though Ross (1952), using Neoprene, was able in some cases to fill the collecting ducts from the pelvis. As a result of these criticisms and observations the author has re-examined the problem and modified his view concerning the nature of the openings of the ducts of Bellini into the renal pelvis.

In an eleven-month-old baby that had died of a syndrome of gastro-enteritis and bronchitis the kidneys still showed a complete picture of uric acid infarcts in spite of there never having been any evidence of kidney impairment.

As the study was carried out using serial sections of complete kidneys the morphology of the pyramids could be examined in different directions, the observations made falling into two groups.

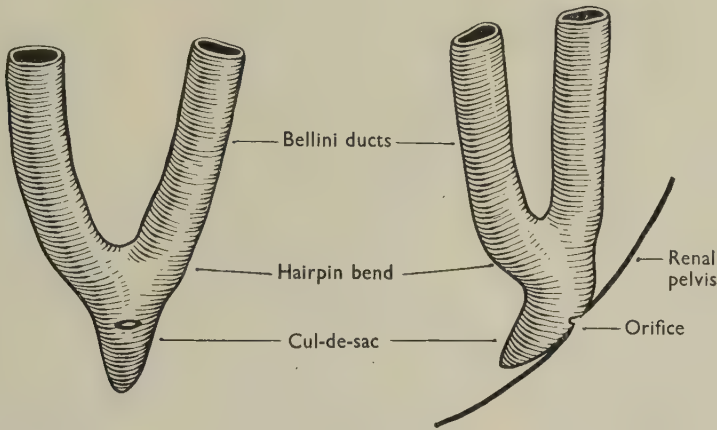
In the first group the pyramids were studied employing serial sections cut parallel to their main direction, and by pursuing their course with serial photographs it could be seen how the Bellini ducts joined to form a hairpin bend, coming into contact with the pelvic epithelia which appeared to cover the orifice of the duct forming a kind of operculum (Pl. 1, figs. 2-7). Only in two sections was there a small free communication present between the duct and the renal pelvis, but after examining some hundreds of sections no definite picture had emerged regarding the shape, size and structure of the communicating orifice.

In the second group the pyramids were studied using transverse sections vertical to their main axis, and this was indeed rewarding. These serial sections showed how some small tubes joined together to form two main ducts, these in turn joining together to produce the 'hairpin bend' which, travelling in a radial direction, came in contact with the pelvic epithelia and opened into the renal pelvis by a lateral and very small orifice (Pl. 1, figs. 8-11). The walls of the Bellini duct then joined together, and the rebuilt duct buried itself in the tissue of the papillae ending in a conical cul-de-sac, this being confirmed in the next section by the complete disappearance of the duct.

This lateral opening of the Bellini duct had an approximate measurement of  $60 \times 20 \mu$ , that is the shape of an elongated orifice.

To visualize the morphology of the Bellini duct in a more schematic manner two illustrations were made (Text-fig. 1) to show the lateral position of the orifice in relation to the hairpin bend and the cul-de-sac ending. The position of the latter explains the doubt expressed in the preliminary communication as to the presence of openings in the Bellini ducts.

The form of the Bellini duct orifices makes the hypothesis feasible that they may have the function of preventing a back flow from the renal pelvis of urine accumulated there under pressure. One finds a similar arrangement in other parts of the urinary system such as the ureteric orifice in the bladder, as well as in other excretory systems like the Hasner valve in the lacrimal duct.



Text-fig. 1. Schematic drawings showing the structure of the Bellini duct.

The narrow opening of the Bellini duct and its structure, as described in this study, adds to the understanding of the selective hold-up of uric acid in the foetal kidney, a finding so often observed by the pathologist.

The appearance of a cul-de-sac structure in the Bellini duct end also suggests that if some mineral deposits are precipitated there it may be difficult to eliminate them, and may perhaps be the starting-point of a sterile urinary calculus (*Lancet*, 1954). At the same time, a blockage or non-development of this orifice would result in a cystic condition of the kidney.

#### SUMMARY

The existence of a narrow elongated orifice is described at the side of the 'hairpin bend' of the Bellini duct, the end of which finishes in a small conical cul-de-sac. Its presence and shape helps to explain the finding of uric acid infarcts in the renal pyramids of the recent-born child.

The use of transverse serial sections of the tip of the renal pyramid is recommended as the most rewarding method of studying the orifices of the Bellini ducts.



Thanks are due to the Medical Staff of Booth Hall Hospital, The Duchess of York Hospital for Babies and Monsall Hospital for the use of their material, and to Mr T. Walsh and the team of technicians who, under his direction, cut more than 4000 serial sections. Thanks are also due to Mr W. Taylor, C.R.S.I., for the animal specimens, and to Miss J. Perry for her sketches.

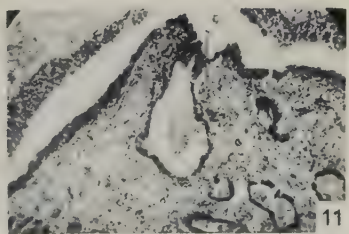
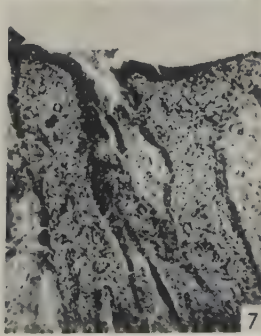
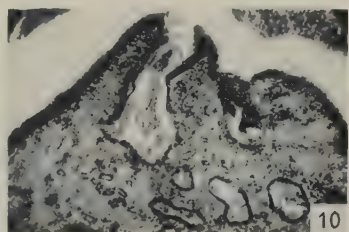
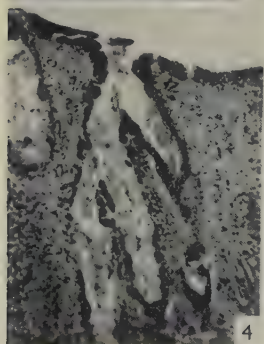
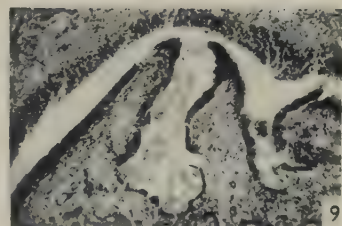
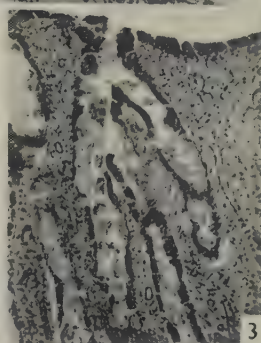
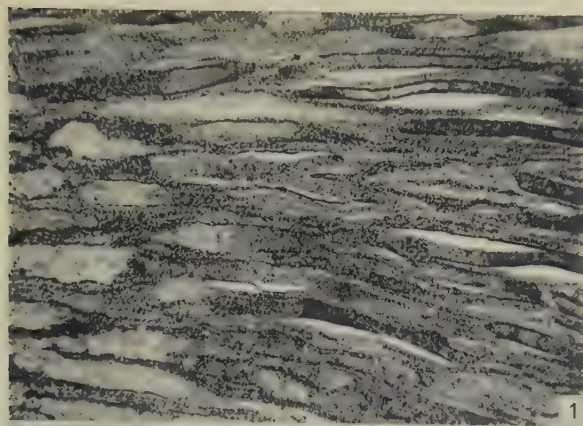
The research was made possible by a grant from the Endowment Fund of Booth Hall Hospital.

#### REFERENCES

- DURAN-JORDA, F. (1953). The renal ducts of Bellini. Observations in new-born babies. *Lancet*, **1**, 727.
- JONES, C. H. & REWELL, R. E. (1954). Correspondence. *Lancet*, **2**, 291.
- Lancet* (1954). Editorial, **2**, 1009.
- ROBSON, S. M. (1954). Personal communication.
- ROSS, J. A. (1952). Studies in pyelorenal backflow. *Brit. J. Urol.* **24**, 27.
- ROSS, J. A. (1954). Correspondence. *Lancet*, **2**, 758.
- THUNG, P. J. (1954). Correspondence. *Lancet*, **2**, 658.

#### EXPLANATION OF PLATE

- Fig. 1. Kidney of recent-born child. Collecting tubes full of uric acid precipitate and eosinophilic material.
- Figs. 2, 3. Serial parallel section showing the formation of a hairpin bend.
- Figs. 4, 5. Serial parallel section showing pelvic epithelia still covering part of the orifice, suggesting an operculum.
- Figs. 6, 7. Serial parallel section showing small free communication between the Bellini duct and the renal pelvis.
- Fig. 8. Transverse serial section showing hairpin bend.
- Fig. 9. Transverse serial section showing orifice.
- Figs. 10, 11. Transverse serial section showing rebuilding of the Bellini duct.







## QUANTITATIVE ANALYSIS OF CELL TYPES IN MAMMALIAN NEO-CORTEX\*

By N. L. MITRA†

*Department of Anatomy, University College, London*

### INTRODUCTION

The classification of neurons into various morphological types has played an important part in the investigation of the structural organization of the cerebral cortex. Such classifications have mainly been based on various modifications of the Nissl and Golgi methods of staining. Using the Nissl method, which stains the cell body and the proximal parts of the larger dendrites of a neuron only, the cortical neurons have been classified, usually into pyramidal, granular and fusiform types, by a number of investigators; to mention only a few of the more recent, Economo & Koskinas (1925), Ngowyang (1937) and Walker (1940). A classification by this method cannot be regarded as satisfactory since only the shapes of the cell bodies are considered while the dendritic and axonal organization is ignored.

Using his own method of staining and on the basis of the axonal ramification, Golgi classified the neurons into two primary groups, type I and type II cells; he further subdivided each group into pyramidal, fusiform and polygonal or stellate types depending on the mode of origin and ramification of the dendrites from the perikaryon. This plan was elaborated by subsequent workers, and at least sixty different types of neurons have been recognized in the cortex of mouse (Lorente de Nó, 1922). Although the value of such qualitative descriptions cannot be denied, adequate information on the relative proportions of the cell types and their precise mode of distribution in the cortex is lacking. For example, the structure of the human visual cortex is apparently fundamentally the same as that of the mouse with regard to the diversity of the cell types (Lorente de Nó, 1934), but a quantitative analysis may, nevertheless, reveal striking differences. Such a quantitative method is clearly valuable for comparing not only the organization of different cortical zones of the same species of animals but also of similar areas in animals of different habits.

The present paper gives estimates of the proportions of cells with various shapes that appear in different parts of the cortex. In Golgi-Cox preparations three different groups of neurons with distinctive types of dendritic organization can be recognized. In one group (the pyramidal cells) each neuron possesses a long apical dendrite and several basal ones, in the second group (stellate cells) the dendrites originate from all parts of the surface of the perikaryon while the third, forming

\* This work was the subject of a dissertation approved for the degree of Ph.D. in the University of London and was aided by grants from the Rockefeller Foundation and the State of Bihar, India. The thesis contains the full statistical data which form the basis of the present paper.

† Present address: Department of Anatomy, Medical College, Darbhanga, Bihar, India.

a small minority, consists of fusiform neurons with a dendrite originating from each pole of the cell body. These cell types are not arranged at random but show patterns of distribution varying in different zones of the cortex.

#### MATERIAL AND METHOD

##### *Animals and functional areas*

Three cats, four rabbits and one monkey (*Macaca mulatta*) were used for observations on adult animals. A limited investigation was also made on the visual area of two young rabbits, 10 and 17 days old, on one kitten 6 weeks old and on the prefrontal cortex of a human subject taken at lobotomy. Of the twenty-six sets of observations, six were on the visual, four on the motor and two on the somato-sensory areas of cats, eight on the visual area of rabbits, two each on the visual and motor areas of monkey (*Macaca*) and one each on the parastriate area of monkey and the prefrontal cortex of man.

Although the histological localization of most of the functional areas on which the present observations were made is fairly easy and accurate, the visual area of rabbits and the somato-sensory area of cats provided some difficulty. The cyto-architectural maps compiled by various authors show considerable differences in the localization of these two areas. Hence, the results of stimulation, ablation and other electro-physiological experiments were also considered when the areas for the present investigation were identified. Only that region within a functional area of the cortex was selected for observation over which the results obtained by the different methods, histological and physiological were in accord.

Block staining by a modification of the Golgi-Cox method was adopted (Sholl, 1953).

##### *Criteria for classification of neurons*

The neurons have been classified into pyramidal, stellate and fusiform or spindle-shaped types, depending on the shape of the cell bodies and on the mode of origin and course of the dendrites. This classification does not in any way take into account the axons of the neurons. Consequently, all the three types of neurons include both Golgi type I and Golgi type II cells.

A typical pyramidal cell is defined as a neuron possessing a conical perikaryon with apical and basal dendrites. The apical dendrite is directed towards the pial surface; it may or may not reach the molecular layer but usually extends for a greater distance than the basal dendrites and is also generally thicker at its origin. The basal dendrites originate from the basal angles and the basal surface of the perikaryon and extend horizontally or obliquely upwards and downwards. The lateral surfaces of the perikaryon, extending between the apical and the basal angles, are devoid of dendrites and this criterion together, with the presence of an apical dendrite, forms a very important distinguishing characteristic of a pyramidal cell. In some cases the perikaryon of a pyramidal cell, instead of being conical, is of ovoid shape and hence does not possess a lateral angle. In such cases, the basal dendrites originate from the basal surface and the lower half of the lateral surface of the cell body, leaving the upper half or more free of dendrites.

Besides these typical cells, certain variations are met with. Thus the large pyramidal cells, such as the Betz cells and the solitary pyramidal cells of Meynert, sometimes possess a few dendrites that originate from the lateral surfaces of conical perikarya, but the configuration of such neurons as a whole leaves no doubt of their pyramidal nature. Another type of pyramidal cell possesses a relatively fine and short apical dendrite, but the cell body is conical and the absence of dendrites on its lateral surfaces renders the classification easy. Sometimes a pyramidal cell is orientated in an inverted manner, that is to say, the 'apical' dendrite is directed towards the white matter of the cortex and the basal surface with its dendrites is directed towards the pial surface of the cortex.

Stellate cells possess spherical, ellipsoidal or polygonal perikarya. The dendrites originate from all parts of the surfaces of the cell body and ramify uniformly around it. Usually all the dendrites are of uniform size at their origin and their lengths are almost the same. In exceptional cases, however, the dendrite directed towards the pial surface of the cortex runs for a longer distance and is appreciably thicker than the rest of the dendrites.

The fusiform cells are situated with their long axes usually parallel but occasionally vertical to the pial surface. They are bipolar and one dendrite originates from each pole of the cell body.

As the results will show, it is possible in this way to classify nearly but not quite all the cells of the cortex; a few are more complex and do not come under any of the three categories. Certain technical limitations also render some of the cells unclassifiable; in the course of sectioning, a few of the cells lose their processes in such a way as to make classification difficult and sometimes the neurons are partially superimposed on each other. In other cases the ramifications of the dendrites of a neuron obscures the details of an adjacent one. About 15–20 % of the neurons in some of the samples had to be left unclassified, but it must be emphasized that neurons possessing shapes other than the three main types formed only a small fraction of these cases.

#### *Method of observation*

On the basis of these criteria the neurons within a strip of cortex belonging to any one of the cortical areas studied were typed and their vertical distances from the pial surface measured. The positions of all the unclassified neurons were noted in order to determine whether they were distributed at any particular depth of the cortex. Owing to the selectivity of the staining method the number of neurons stained per unit volume of cortex varies in different animals and results expressed in terms of the absolute numbers of the various cell types would obviously be misleading. The purpose of the present analysis was, therefore, to determine the mode of distribution of the various cell types and their relative proportions only; consequently, no attempt was made to maintain a constancy in the width of the various strips within which the counts were made. A sample of about 300–350 neurons was considered adequate for each set of observations. In the motor area of the various animals and in the visual area of young animals, however, the cell density in the stained preparations is always rather poor and a sample of 300 cells could not be



taken without the risk of a part of it being obtained from a different functional area. In such cases, each sample had to be reduced to about 200 cells.

The portions of cortex for examination were restricted to those having a relatively plane pial surface, since the measurement of the vertical distances of the neurons below the pial surface over a wide zone of the cortex possessing a sharp curvature cannot be made without considerable error and the shapes of the neurons at highly curved regions of the cortex are altered as a result of mechanical factors.

The vertical distance of a neuron from the pial surface was measured between the deeper surface of the pia mater and the upper edge of the perikaryon of the neuron. The constriction usually present at the beginning of a dendrite was accepted as the limit of the perikaryon. In some cases, however, no such constrictions are present and the cell body tapers gradually to be continued into a dendrite. In such cases, the decision had to be arbitrary.

It was found that the unclassified cells have no special zones of concentration in the cortex. The fusiform cells are very few in number. The pyramids and the stellates form the two main cell types (according to this mode of classification) and constitute the main features of interest. Their patterns of distribution have been examined by the construction of superimposed histograms representing the frequency of each cell type in 100  $\mu$  depth strips below the pial surface of the cortex; the relative proportion of each variety of neurons has been expressed as a percentage of the total number of classified neurons.

#### *Statistical considerations*

The Golgi-Cox method of staining is highly selective, and the selectivity is known to vary from animal to animal as evidenced by the number of cells stained per unit volume of the cortex. Moreover, this variation may not be uniform over the whole depth of the cortex. A preference for a particular cell type in some localized regions might lead to an apparent change in the patterns of distribution and the relative proportions of the various cell types. The need for a statistical method for testing the consistency of the results becomes obvious.

The distribution of the cells in 100  $\mu$  depth strips below the pial surface in various samples from the same functional area of animals belonging to the same species was tested for consistency by the  $\chi^2$  method. The samples from the same animal proved to be always consistent, but those from different animals were slightly but significantly different. However, examination of the actual frequency distributions of the pyramidal and stellate cells and the contributions made by the different groups to the total value of  $\chi^2$  show that the differences are not due to any general changes in the form of the distributions but rather to slight differences at one or two depths only. The relative proportions of the two cell types differ only slightly in different animals of the same species (Table 1). There is thus reason to think that in this series variations in the selectivity of the staining do not cause any serious differences either in the manner of distribution or in the relative proportions of the pyramidal and stellate cells in a particular functional area of animals within the same species.

## RESULTS

*The visual area of Macaca*

The distribution of the various cell types were examined in strips  $100\mu$  wide taken throughout the depth of the cortex in two samples from the same animal. The unclassified cells form about 16–20 % of the total number of the neurons counted. They are, however, randomly distributed throughout the depth of the cortex, and

Table 1. *Relative proportions of the cell types in certain cortical areas of different mammals*

Animal and area		Cell types expressed as percentages of the total number of classified neurons		
		Pyramidal	Stellate	Fusiform
Monkey visual	4a	54	44	2
	4b	51	47	2
Cat visual	190a	62	34	5
	190b	63	34	3
	190c	57	37	6
	236	63	33	5
	307	61	37	2
	244 (6 weeks)	66	31	3
Rabbit visual	2366a (adult)	66	32	2
	2366b (adult)	69	28	3
	2357a (adult)	65	32	3
	2357b (adult)	64	32	4
	2436 (adult)	69	29	2
	2487 (adult)	65	30	6
	2303 (17 days)	74	23	3
	2557 (10 days)	85	13	2
Cat somatosensory	307a	64	35	1
	307b	62	36	2
Monkey motor	4a	75	21	4
	4b	73	23	4
Cat motor	273a	84	10	6
	273b	86	8	6
	265a	86	8	5
	265b	84	12	5
Human prefrontal	—	72	26	2

their proportion varies with the density of neurons at each depth strip. The fusiform cells, on the other hand, form an insignificant proportion accounting for only 2 % of the total number of classified neurons. The pyramids and the stellates therefore form the main cell types and, as the two samples were found to be statistically consistent, the distributions have been added together and superimposed histograms constructed (Tables 1 and 2, and Fig. 1).

The pyramids are mainly distributed in the upper and the lower thirds of the cortex, whereas the stellate cells are mainly concentrated in its middle one-third; within a zone of about  $700\mu$  ( $600\mu$ – $1300\mu$  below pial surface) they are more numerous than the pyramidal cells. For convenience of assessment of the relative proportions of these two cell types at different levels, the cortex of areas showing such a specific distribution of stellate cells has been arbitrarily divided into three zones, a 'stellate zone' where there are more of these cells than pyramids, a 'supra-

stellate zone' lying above and an 'infra-stellate zone' lying deep to the stellate zone, both of which contain more pyramids than stellates. There are very few stellate cells in the supra- and infra-stellate regions of the visual cortex of the monkey and they form only 9 and 8 % respectively of the total number of these two cell types in these regions. Moreover, the peak of the stellate cell distribution reaches a higher level than that of the pyramids, and stellate cells form an average of about 45 % of the total number of classified neurons (Table 2).

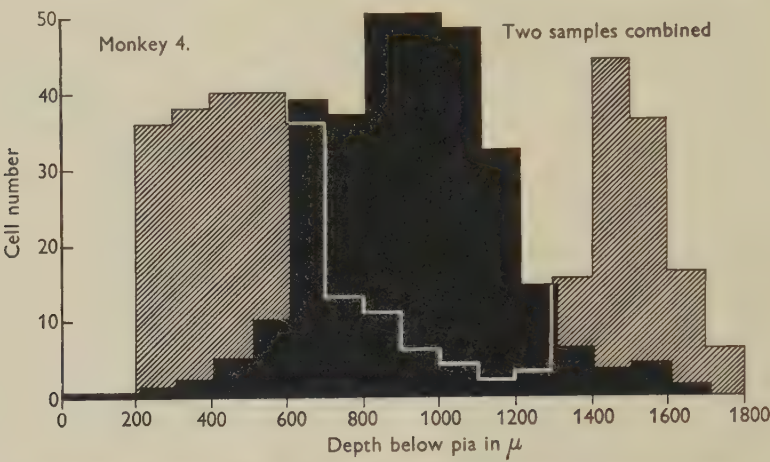


Fig. 1. Distribution of pyramidal and stellate cells in the visual cortex of *Macaca*. Shaded area: distribution of pyramidal cells. Black area: distribution of stellate cells.

Table 2. *Relative percentages of the cell types in various cortical areas of different mammals*

Animal and area	Cell types expressed as percentages of total number of classified neurons		
	Pyramidal	Stellate	Fusiform
Rabbit visual (adult)	66.0	31.0	3.0
Cat visual (adult)	60.0	35.0	5.0
Monkey visual (adult)	52.0	45.0	3.0
Cat somatosensory (adult)	63.0	35.0	2.0
Monkey motor (adult)	74.0	22.0	4.0
Monkey parastriate (adult)	66.0	29.0	5.0
Cat motor (adult)	85.0	9.0	6.0
Human prefrontal (adult)	72.0	26.0	2.0
Kitten visual (6 weeks)	66.0	31.0	3.0
Rabbit visual (17 days)	74.0	23.0	3.0
Rabbit visual (10 days)	85.0	13.0	2.0

*The visual area of the cat*

The proportion of unclassified cells varied from 3 to 13 %, distributed without any preference for any particular depth of the cortex. The samples from the different adult animals show only minor variations in the distribution of the cell types, mainly owing to thickness differences in the depth of cortex and slight variations in the zone of the stellate cell concentration. The histograms in Fig. 2 show that the patterns of stellate and pyramidal cell distributions are similar in the three adult



cats.  $\chi^2$  tests showed the presence of significant differences but these are not of great magnitude (Table 1).

The stellate cells are concentrated in the middle of the cortex over a zone of 400–500  $\mu$  thick and slightly narrower than that found in the monkey (700  $\mu$  in the latter). This concentration is attained more gradually and the stellate cells form on the average, about 15 % of the total number of cells in the 'suprastellate' zone. The peak of the stellate cell distribution does not generally exceed that of the pyramids but both are nearly of the same height. The stellate cells form an average of 35 % (Table 2), this is significantly less than in the case of the monkey (45 %).

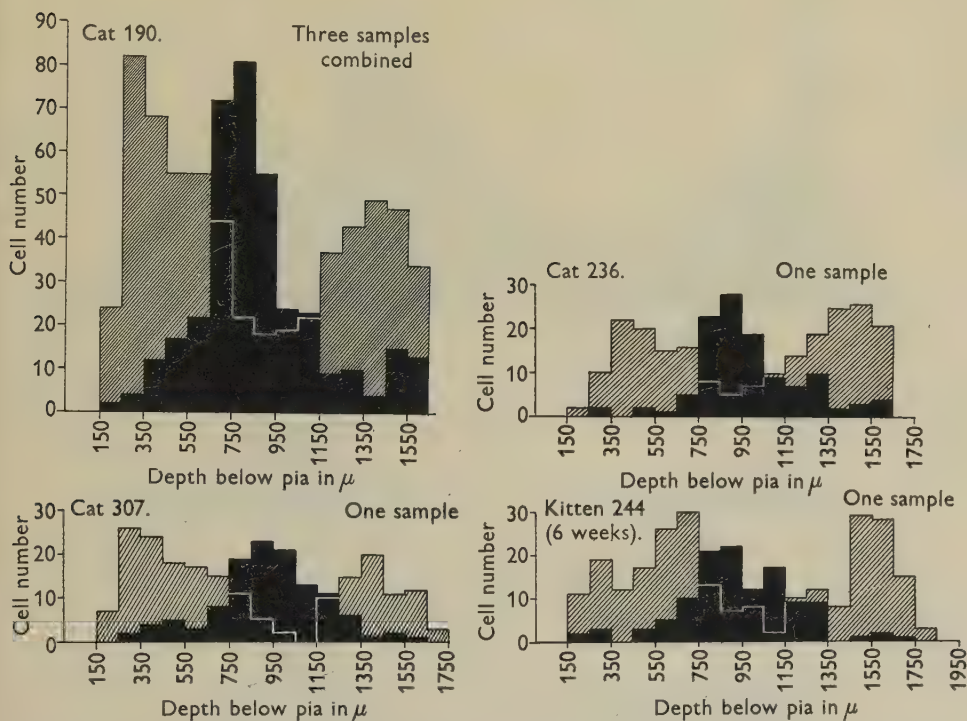


Fig. 2. Distribution of pyramidal and stellate cells in the visual cortex of the cat. Cat 190: three samples combined. Cat 236: one sample. Cat 307: one sample. Kitten 244 (6 weeks): one sample. Shaded area: distribution of pyramidal cells. Black area: distribution of stellate cells.

In the 6-week-old kitten, the distribution patterns of the stellates and pyramids are similar to the adults, but there are fewer stellate cells (31 %) (Table 2), and the peak of the stellate cell distribution is at a lower level than that of the pyramids.

### *The visual area of the rabbit*

The unclassified and the fusiform cells form about 9–18 and 4 % of the samples respectively, and the former are again found to be distributed in a random manner at all depths of the cortex. The difference between the pair of samples from each of the two adult rabbits was found to be statistically insignificant, but comparisons

between the samples from the different adult animals were significantly different. The causes giving rise to such differences have been discussed earlier (p. 469), and no consistent pattern could be detected in the distribution of these differences.

The patterns of distribution of the pyramidal and stellate cells are represented in a series of histograms in Fig. 3, each of which represents the distribution in one animal. In spite of possible differences suggested by the  $\chi^2$  test, these patterns appear to be very similar in the adult animals. The zone of stellate cell concentration

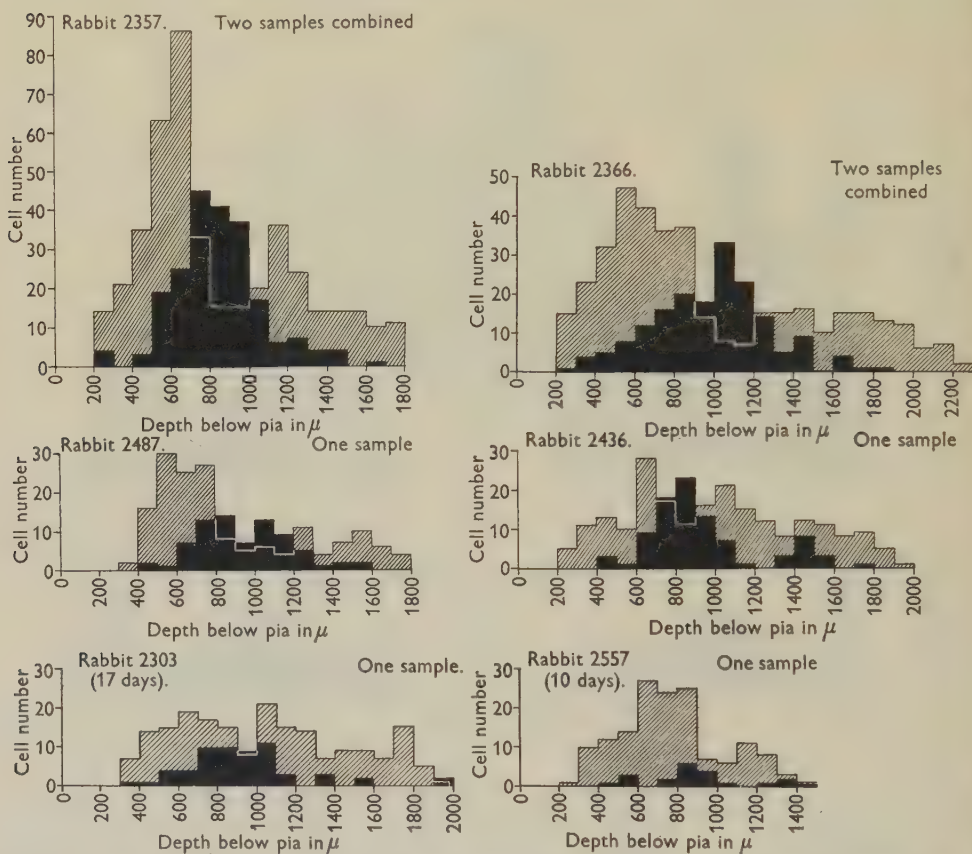


Fig. 3. Distribution of pyramidal and stellate cells in the visual cortex of the rabbit. Rabbit 2357: two samples combined. Rabbit 2366: two samples combined. Rabbit 2487: one sample. Rabbit 2436: one sample. Rabbit 2303 (17 days): one sample. Rabbit 2557 (10 days): one sample. Shaded area: distribution of pyramidal cells. Black area: distribution of stellate cells.

is narrower in this species than in the monkey or cat and occupies only a zone 300–400  $\mu$  thick compared with 700  $\mu$  in the monkey and 400–500  $\mu$  in the cat visual area. Beyond this zone the number of stellate cells diminishes more gradually than in the latter two species of animals and becomes 19% of the total number of pyramids and stellates in the 'suprastellate zone'. The peak of the stellate cell distribution is at a significantly lower level than that of the pyramidal cells, showing that their concentration is relatively less in rabbits than in cats and

monkeys. The percentage of the stellate cells varies between 28 and 32 % with an average of approximately 31 % (Table 2).

Examination of the stellate cell distributions in young animals (Fig. 3) leaves no doubt that they are very different from the adults. The stellate cells are concentrated in a region of the cortex where in the adults they outnumber the pyramids but they form only approximately 23 % of the classified neurons in the 17-day-old animal and 13 % in the 10-day-old rabbit in comparison with 31 % in the adults; moreover, at these early ages, there are more pyramids than stellates at all depths of the cortex.

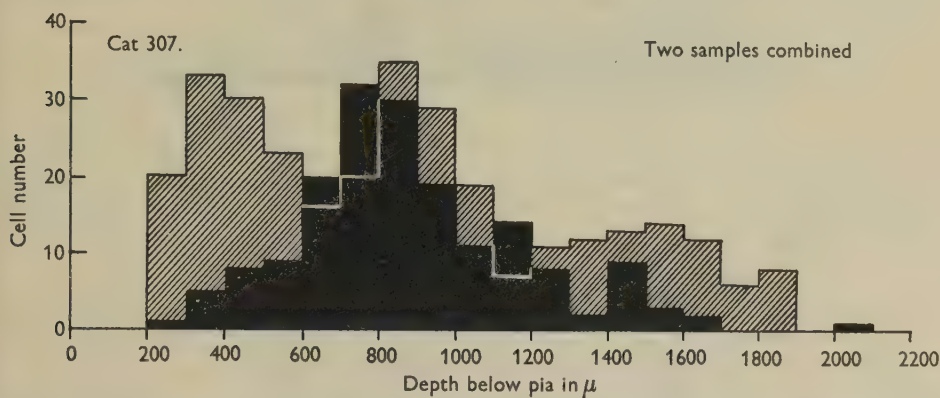


Fig. 4. Distribution of pyramidal and stellate cells in the somatosensory cortex of the cat. Cat 307: two samples combined. Shaded area: distribution of pyramidal cells. Black area: distribution of stellate cells.

#### *The somatosensory area of the cat*

Two sets of observations were made in one animal and the cell distributions in the two samples were found to be statistically consistent. They have been added together and are represented in Fig. 4. The stellate cells are seen to be distributed in a way similar to those of the visual area of the other species of animals examined and their zone of predominance is almost the same as that of the visual cortex of cat. This predominance however, is less well marked as can be seen from a comparison of the histograms in Figs. 4 and 2. The concentration is attained more gradually, the stellate cells form about 18 % of the neuron population in the supra-stellate region as compared with about 15 % in the visual area of cats, but the relative proportion of these cells in the whole depth of the cortex is the same in both areas.

#### *The parastriate area of Macaca*

Fifty-two cells out of a total of 357 could not be classified, forming about 15 % of the total sample and only fourteen cells could be classified as belonging to the fusiform group. The superimposed histograms in Fig. 5 show that the stellate cells are distributed more uniformly throughout the whole depth of the parastriate than of the striate cortex, with a slight preference for the more superficial regions. The selective mode of distribution of the two main cell types seen in the primary receptive areas is totally lacking. Another striking difference is that at every depth



of the cortex below the pial surface the stellates are fewer in number than the pyramids; they form only about 29 % (Table 2) of the classified cells, as compared with 45 % in the visual area of the same animal.

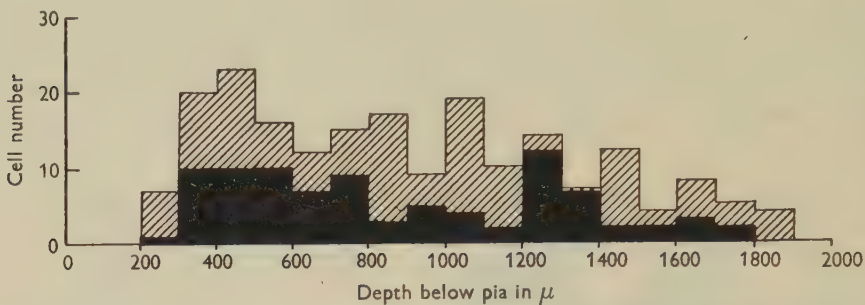


Fig. 5. Distribution of pyramidal and stellate cells in the parastriate cortex of the monkey. Monkey 4: one sample. Shaded area: distribution of pyramidal cells. Black area: distribution of stellate cells.

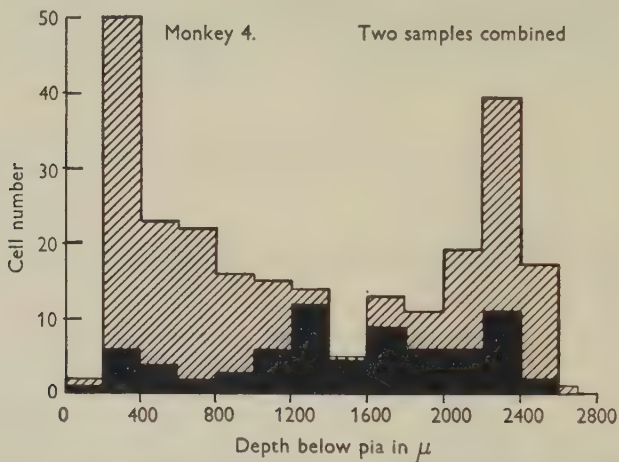


Fig. 6. Distribution of pyramidal and stellate cells in the motor cortex of the monkey. Monkey 4: two samples combined. Shaded area: distribution of pyramidal cells. Black area: distribution of stellate cells.

#### *The motor area of the monkey*

Only 17 % of the neurons could not be classified and, unlike the other areas examined, these are slightly more frequent in the deeper parts of the cortex. This is explained by the fact that in the wall of a sulcus the corticopetal and corticofugal nerve fibres take a sharp bend as they enter or leave the grey matter and thus tend to modify the shapes of the neurons; the result of these mechanical factors makes their classification more difficult.

The pattern of distribution of the stellate cells is similar to that of the parastriate area of the same animal, in that they are distributed uniformly throughout the whole depth of the cortex and at every depth they are outnumbered by the pyramidal cells.

There is a slightly greater frequency of the stellate cells in the deeper parts of the cortex and their proportion is slightly less, forming about 22 % (Tables 1 and 2) of the total number of classified neurons compared with 29 % in the parastriate area.

*The motor area of the cat*

Three samples were taken from the caudal wall of the cruciate sulcus, two from one cat and one from another. All the distributions were found to be statistically consistent and hence these distributions have been added. Those of a fourth sample derived from the rostral wall have been considered separately on account of the

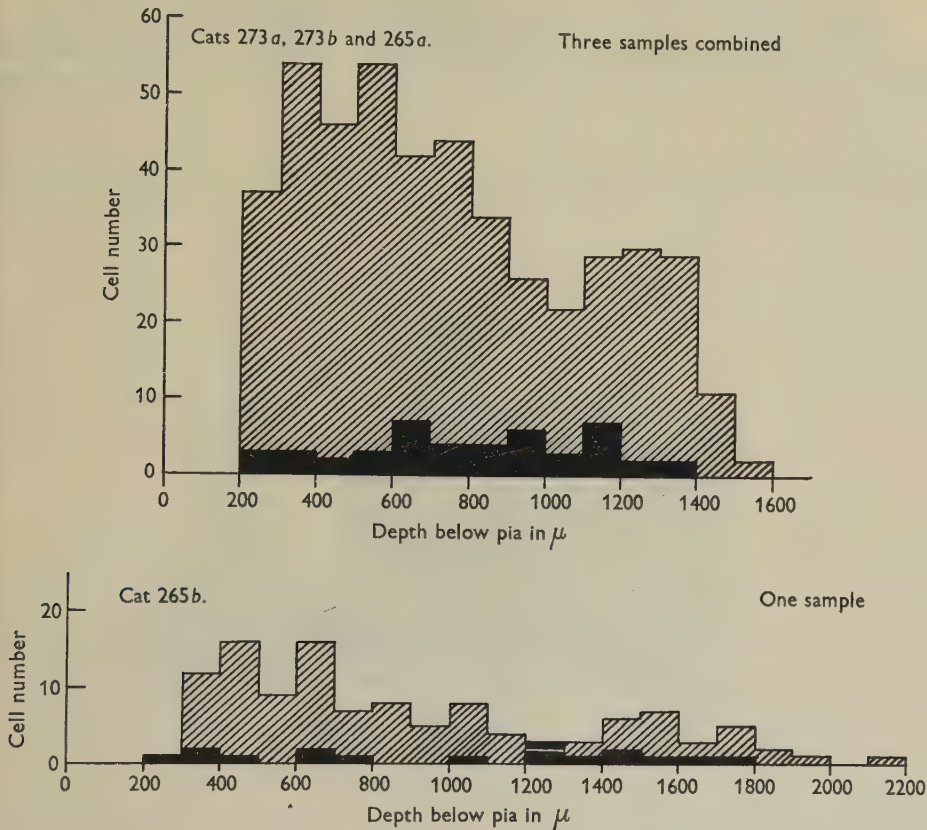


Fig. 7. Distribution of pyramidal and stellate cells in the motor cortex of the cat. Cats 273a, 273b and 275a; three samples combined. Cat 265b; one sample. Shaded area: distribution of pyramidal cells. Black area: distribution of stellate cells.

great differences in the thickness of the cortex of the rostral and caudal walls of the sulcus. The unclassified cells formed 15 % of the samples. The histograms in Fig. 7 show that the stellate cells, as in the case of the monkey, are distributed uniformly at all depths of the cortex, but only 9 % of the cells are stellate (Table 2).

*The prefrontal cortex of man*

The cell distribution in this area of the human cortex is very similar to that found in the parastriate area of monkey, and the relative proportions of the cell types are almost the same in both areas (Table 1 and Fig. 8). 16 % of the cells were unclassified.

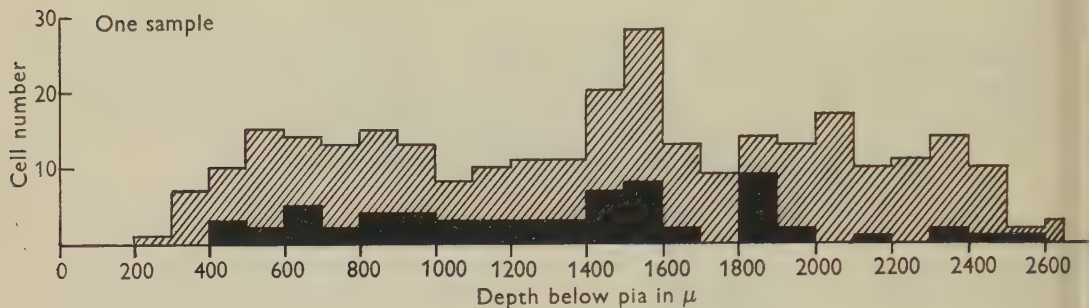


Fig. 8. Distribution of pyramidal and stellate cells in the prefrontal cortex of man. One sample. Shaded area: distribution of pyramidal cells. Black area: distribution of stellate cells.

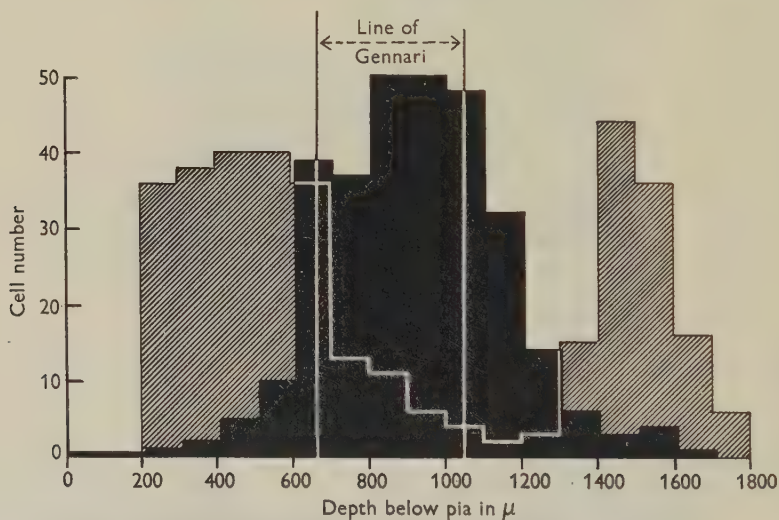


Fig. 9. Distribution of the stellate cells in relation to the line of Gennari in the visual cortex of the monkey.

*Relationship of the specific afferent fibres with stellate cell distribution in the visual area of the monkey*

The line of Gennari, where most of the specific afferent fibres from the lateral geniculate body ramify and terminate is very prominent in the monkey and can easily be identified on sections stained by Nissl or silver methods. In order to investigate the spatial relationship of the stellate cells to the specific visual afferents, the width of the line of Gennari and its depth below the pial surface of the cortex



were measured on sections stained with a silver (Bielschowsky) method. These measurements were made on the opposite (right) hemisphere of the same macaque whose left hemisphere had been used for cell typing. The relationship of these afferents to the area of stellate cells distribution is shown in Fig. 9, in which the two white lines represent the position and extent of the line of Gennari as measured on silver preparations.

It is clear from this figure that the line of Gennari corresponds closely to the area of distribution of the stellate cells. Although some of these stellate neurons are situated beyond the limits of Gennari's line, the majority are contained within them; in other words, within the zone of termination of the specific afferents.

## DISCUSSION

### *Consistency of the Golgi-Cox method*

The Golgi technique provides more information about the processes of neurons than any other method, but it has been little used for quantitative work. This has perhaps been due to fears that there may be wide variations in the proportions of cells that are stained. The present figures show that with the Golgi-Cox method used the proportions of pyramidal, stellate and fusiform cells do not differ greatly in different samples of one cortical area in the same animal, or between different individuals of the same species. Nevertheless, there are consistent differences between areas. This result does not of course show that the cells actually occur in the recorded proportions. It is impossible to prove that there are not systematic differences in the proportions of the various types that are stained. There is, however, no evidence of such selectivity in these figures and if it exists it must be relatively constant.

### *Horizontal cells and fusiform cells*

Very few horizontal cells of Cajal have been observed either in the adults or in the young animals and the few that have been seen resemble the stellate cells more closely than the Cajal cells proper. It is to be noted that most of the investigations reporting the presence of horizontal cells have been carried out on embryonic cortex or in very young animals in which these cells have not differentiated to the full extent (Ramón y Cajal, 1891, 1893; Retzius, 1891, 1893, 1894; Veratti, 1897). Further evidence of the relative immaturity of these horizontal cells at the time of birth is provided by Ramón y Cajal himself (1900-6) who found that some of the processes of the horizontal cells undergo atrophy during postnatal maturation of the cortex. It is therefore probable that these cells represent a transitional phase and that later on they change their shapes through the atrophy of some of the processes and perhaps by the growth of fresh ones till there are very few, if any, horizontal cells left in the adult cortex.

In agreement with a number of previous observers, very few fusiform cells are found in regions of cortex possessing comparatively plane surfaces. They are most abundant on the crowns of the gyri and at the depths of the sulci, and are mostly confined to the deeper parts of the cortex. It seems probable that some of the pyramidal and a few of the stellate cells in these regions are compressed in a vertical or in a horizontal direction.

*Afferent fibres as a determinant of stellate cell organization in the cortex*

The intimate spatial relationship between the stellate cells and the termination of the specific afferent fibres in the visual cortex suggests a close correlation between the number of stellate cells and the density and distribution of the afferent plexus in the cortex.

In cortical areas that are not primarily sensory, for example in the parastriate area of the monkey and the frontal cortex of man, the stellate cells are distributed almost uniformly throughout the whole depth of the cortex and this may be associated with the fact that the afferent fibres in these regions do not form a localized plexus but terminate at all cortical levels (Lorente de Nó, 1949).

In the series rabbit, cat and monkey there is a progressive increase in the proportion of the stellate cells in the visual area, together with their more restricted distribution to this area. This may be correlated with the facts that the termination of the specific afferents in the visual cortex of cat is less diffuse and more branched than in rabbits (O'Leary & Bishop, 1938), and that the line of Gennari is much wider and more prominent in monkeys than in cats. This may be correlated with the mode of organization of the afferent plexus in these animals.

Polyak (1932) showed that the afferent plexus in the somatosensory area of the monkey is more diffuse than in its visual area. If this is also true of cats, it may well explain the more diffuse distribution of the stellate cells in the somatosensory area of these animals.

There is therefore a strong suggestion that the density and the mode of distribution of the afferent fibres may be related to the relative proportion and the pattern of distribution of the stellate cells.

*The comparative development of the cell types in the cortex*

From a series of non-quantitative comparative studies on various mammals Mott (1907) advanced the theory that a more highly organized visual cortex is correlated with a more well-developed layer of supragranular pyramidal cells and in this he was supported by Watson (1907) and Ariëns Kappers (1909). Although no statement of a positive nature was made, it seems that the statement 'the development of the supragranular pyramidal layer' implies not only a more complete segregation of the supragranular pyramidal cells from the granular cells but a progressive increase in the relative proportion of the pyramidal cells as well. The present quantitative findings on the visual area (Table 2) show that although there is a progressive increase of separation of the suprastellate pyramidal cells from the stellate cells in the series rabbit, cat, monkey, the relative proportion of the stellate cells, instead of decreasing, shows a definite increase in the same order. In other words, a progressive concentration of the stellate cells runs parallel with an increase of their proportion of the total cell content.

*Progressive increase in the proportion of stellate cells with post-natal development*

The progressive increase in the relative proportion of the stellate cells with post-natal development has not been reported earlier. This increase may be attributed to

several factors: (a) some of the stellate cells at birth may exist in the cortex as neuroblasts which are not stained by the Golgi-Cox method and may gradually develop into mature neurons, (b) some of the pyramidal cells may be transformed into stellate cells by the growth of secondary dendrites from the cell bodies and by the attenuation of the apical dendrites, and (c) the Golgi-Cox method of staining may be less selective towards the stellate cells in young animals, causing an apparent reduction in the proportion of the stellate cells stained. The possibility, however, of mitosis of neuroblasts in the cortex causing an increase in the relative proportion of the stellate cells must be borne in mind though no evidence of such mitosis has been found.

The hypothesis that the Golgi-Cox method stains a smaller proportion of the stellate cells in young animals is not impossible, but appears improbable in view of the fact that in adults it is found to be slightly more selective towards the stellate cells. The number of the cells stained by this method in young animals is much less than in the adults and progressively increases with the growth of the animal. This suggests, but does not prove, that a considerable proportion of the cortical neurons at birth exist in the neuroblast stage (and hence are unstained) and of these a larger proportion belongs to the stellate type. This suggestion is further strengthened by the consideration that the cortical neurons in very young animals appear to be much less developed than in the adults with respect both to dendritic and axonal proliferations. Postnatal growth of axon collaterals and dendrites has been reported by Ramón y Cajal (1900-6), Lorente de Nó (1922) and Conel (1939, 1941, 1947, 1951).

The possibility of some of the pyramidal cells changing into stellate type, remote as it may appear, cannot be disregarded. At the present time the whole question must remain open and await further investigation.

*The possible role of stellate cells in the discrimination of visual patterns*

The ability to discriminate between visual patterns is more highly developed in monkeys than in cats and rabbits, and this situation is closely correlated with the numbers of stellate cells in the striate cortex associated with the region of termination of the specific afferent fibres. Sholl & Uttley (1953) have suggested a possible analogy between this part of the cortex and a machine for pattern discrimination. A machine of this kind demands a number of identical units, the number of which increases with the complexity of the patterns to be discriminated. It would be hazardous to press this analogy too far at the present state of our knowledge, but the design of such a machine suggests that the stellate cells may have properties and connexions comparable with the basic units of these authors.

SUMMARY

1. Estimates were made of the proportions of pyramidal, stellate and fusiform cells in Golgi-Cox preparations of various areas of the cortex of rabbits, cat, monkey and man.

2. The proportions do not differ greatly in different samples from a given area in any one brain nor in different individuals of the same species. There are, however, consistent differences between different 'functional' areas and between each area in different mammals.



3. The proportion of fusiform neurons is very low and horizontal cells are almost or quite absent from the areas examined in adult animals.
4. The stellate cells are mainly concentrated in the middle third of the cortex of the primary sensory areas, whereas in the motor, parastriate and prefrontal cortex these cells are more uniformly distributed throughout the cortex. Their proportion is also greater in the primary sensory areas.
5. There is a progressive increase in the proportion of the stellate cells and the width of the zone of their predominance in the visual cortex of the series rabbit, cat and monkey. There is also a progressive decrease in the same series of the relative proportion of these cells in the cortical region superficial to the zone of predominance of the stellate cells.
6. The zone of termination of the specific visual afferents appears to be highly correlated with the zone of maximum concentration of stellate cells in the visual cortex of monkey.
7. The proportion of the stellate cells increases with the postnatal age in the rabbit.

It is a pleasure to record my indebtedness to Prof. J. Z. Young, F.R.S. for his continued encouragement and criticism throughout this work, and to Mr D. A. Sholl for his advice on the statistical problems involved in this work and on the last part of the paper. My thanks are also due to Miss E. R. Turlington for preparing the histograms and to Mr J. Armstrong, Mr G. Hyde and Miss R. Proudlock for technical assistance.

#### REFERENCES

- ARIËNS KAPPERS, C. U. (1909). The phylogenesis of the palaeo-cortex and archi-cortex compared with the evaluation of the visual neo-cortex. *Arch. Neurol. Psychiat., Lond.*, **4**, 161-173.
- CONEL, J. LE ROY (1939, 1941, 1947, 1951). *Postnatal Development of the Human Cerebral Cortex*. Vols. 1-4. Cambridge, Mass.: Harvard University Press.
- ECONOMO, C. VON & KOSKINAS, G. N. (1925). *Die Cytoarchitektonik der Hirnrinde des erwachsenen Menschen*. Vienna: J. Springer.
- LORENTE DE NÓ, R. (1922). La corteza cerebral del ratón. *Trab. Lab. Invest. biol. Univ. Madr.* **20**, 41-78.
- LORENTE DE NÓ, R. (1934). Studies on the structure of the cerebral cortex. 1. The area entorhinalis. *J. Psychol. Neurol., Lpz.*, **45**, 381-438.
- LORENTE DE NÓ, R. (1949). Cerebral cortex: Architecture, intracortical connections, motor projections. In Fulton, J. F., *Physiology of the Nervous System*, 3rd ed. New York: Oxford University Press.
- MOTT, F. W. (1907). The progressive evolution of the structure and functions of the visual cortex in mammalia. *Arch. Neurol. Psychiat., Lond.*, **3**, 1-48.
- NGOWYANG, G. (1937). Structural variations of the visual cortex in primates. *J. comp. Neurol.* **67**, 89-107.
- O'LEARY, J. L. & BISHOP, G. H. (1938). The optically excitable cortex of the rabbit. *J. comp. Neurol.* **68**, 423-478.
- POLYAK, S. (1932). The main afferent fibre systems of the cerebral cortex in primates. *Univ. Calif. Publ. Anat.* **2**, 1-369.
- RAMÓN Y CAJAL, S. (1891). Sur la structure de l'écorce cérébrale de quelques mammifères. *Cellule*, **7**, 125-176.
- RAMÓN Y CAJAL, S. (1893). Neue Darstellung von histologischen Bau des Centralnervensystem. *Arch. Anat. Physiol., Lpz.* (Anat. Abt.), 1893, pp. 319-428.
- RAMÓN Y CAJAL, S. (1900-6). *Studien über die Hirnrinde des Menschen*. Leipzig: J. A. Barth.

- RETZIUS, G. (1891). Über den Bau der Oberflächenschicht der Grosshirnrinde beim Menschen und bei den Säugetieren. *Biol. Fören. Förh.* **3**, 90–102.
- RETZIUS, G. (1893). Die Cajal'schen Zellen der Grosshirnrinde beim Menschen und bei Säugetieren. *Biol. Untersuch.*, N.F., **5**, 1–8.
- RETZIUS, G. (1894). Weitere Beiträge zur Kenntniss der Cajal'schen Zellen der Grosshirnrinde des Menschen. *Biol. Untersuch.*, N.F., **6**, 29–36.
- SHOLL, D. A. (1953). The dendritic organization of cortical neurons in the cerebral cortex of the cat. *J. Anat., Lond.*, **87**, 387–406.
- SHOLL, D. A. & UTTLEY, A. M. (1953). Pattern discrimination and the visual cortex. *Nature, Lond.*, **171**, 387–388.
- VERATTI, E. (1897). Über einige Struktureingentümlichkeiten der Hirnrinde bei den Säugetieren. *Anat. Anz.* **13**, 377–389.
- WALKER, A. E. (1940). A cytoarchitectural study of the prefrontal area of the macaque monkey. *J. comp. Neurol.* **73**, 59–86.
- WATSON, G. A. (1907). The mammalian cerebral cortex, with special reference to its comparative histology. I. Order Insectivora. *Arch. Neurol. Psychiat., Lond.*, **3**, 49–117.

## THE POSTNATAL DEVELOPMENT OF THE HUMAN CARDIAC VENTRICLES

By E. N. KEEN

*Department of Anatomy, University of Cape Town*

Spigelius (1626) and William Harvey, in the seventeenth chapter of *De Motu Cordis* (1628), made the first known references to the difference between the foetal and postnatal ventricles. There has since been a widespread, if not universal, tendency to believe that in the foetus the right and left ventricles have equal status. The rate at which this relationship changes to a structural and functional balance which characterizes the normal adult heart has attracted little study. The main exception was W. Müller, who published his book *Die Massenverhältnisse des menschlichen Herzens* in 1883. Unfortunately, Müller's approach to the problem was, as will be shown, uncritical in many respects, and his conclusions cannot therefore be supported without modification. Müller has been much quoted, or rather misquoted, as those who have copied his results seem usually to have ignored his printed conclusions. The result is that his work has been held to support widely different statements. Patten (1930) made the most precise of these statements, but this was derived from a quotation of Müller by Gross (1921), whose comment on his predecessor's work was unfortunately misleading.

A fresh investigation seemed therefore necessary. The present study is directed to the weight of the foetal ventricular mass and of its parts, and to the postnatal changes which these undergo. The validity of the common beliefs about the foetal heart will be examined and the possible functional significance of the findings discussed.

### MATERIAL

Two hundred and thirty-seven hearts were examined. At the time they were collected these were all believed to be normal hearts, i.e. neither showing congenital defect nor derived from subjects whose disease processes might have caused ventricular hypertrophy (or atrophy). The specimens were derived from infants and children whose bodies were brought to the Police Mortuary, Cape Town, during 1951 and 1952. The large majority of the deaths were due to natural causes and in these cases the bodies were only brought to the mortuary because no death certificate was available. In the minority an accident such as overlaying was the cause of death. The series as a whole is representative of the less privileged classes living in and around Cape Town.

Thirty-one specimens were classified as from infants prematurely born. In addition, four other specimens (three at the age of 3 months and one at 5 months) were judged, on grounds which will be stated later, to have been derived from infants prematurely born.

The remaining 202 specimens were taken from the bodies of infants which were considered to have been full-term at birth. All except six were accepted as being



normal hearts. The six consisted of five cases of right ventricular hypertrophy, two due to pulmonary tuberculosis and three unexplained, and one case of generalized ventricular hypertrophy, also unexplained. The grounds for the exclusion of these six from the normal series will also be mentioned later.

Classification of the newly born infants into premature and full-term births was naturally easy, but the decision in infants aged 2 months or more was often difficult as it was rare for the parents to know what the weight of the baby had been at birth. In the face of malnutrition and disease it was clearly useless to rely on the weights of the infants, and there appears to be no published guide to the growth in length of infants born prematurely or to the influence of malnutrition on the growth in length of the mature infant under 6 months of age. The decision as to whether a particular infant had or had not been born prematurely therefore often became a matter of judgement on the part of the pathologist concerned, assisted in some cases by the recollections of the parents. These difficulties make it probable that some of the specimens were derived from infants born prematurely, despite efforts to exclude them. On the other hand, some cases may have been excluded in error. The difficulties to which this selection gives rise are discussed below. The infants from which the 231 hearts considered normal were derived were classified as to age in the following way:

Prematurely born

Stillborn	11	} Total at 'birth' 20
Aged 3 days or less	9	
Aged 4 days or more	15	

Full-term infants

Stillborn	15	} Total at 'birth' 36
Aged 3 days or less	21	
Neonatal period	10	
1 month old	13	
2 months old	15	
3 months old	14	
4 months old	14	
5th and 6th months	17	
7th to 12th months	21	
Second year	22	
Third year	10	
Fourth and subsequent years	24	
Total	231	

This classification is so arranged that the mean age of the specimens grouped in the class '2 months old' was approximately 2 months; on the other hand, the designation '5th and 6th months' or 'second year' indicates all specimens from children dying in those periods. The neonatal cases were arbitrarily limited to the ages 4-25 days. The mean age of this group was 11 days with a standard error of 1.9 days. The present

study is particularly directed to the series of full-term infants and children from birth until 3 years. The hearts from stillborn infants and those dying in the first 3 days of life were grouped together as representing the conditions at birth; data relating to them are included in the tables and graphs under the heading 'Birth'.

#### METHOD

*Collection of specimens.* The atria of each heart were freely opened, and as much as possible of the blood and clots washed out from atria and ventricles. The ventricles were not incised. The specimen was labelled with a name and reference number, but age and other details were deliberately not included on the label. In the case of specimens not personally collected this information was not sought until the dissection and weighings had been completed. The specimens were stored in 10 % formol

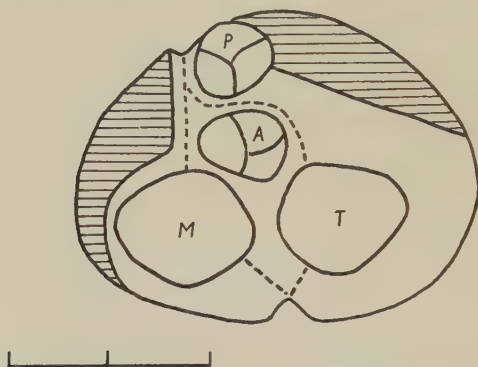


Fig. 1. Stillborn full-term infant, atrial surface of the ventricular mass after removal of the atria and the great arteries. Shading represents undisturbed pericardium. Lines of division indicated by interrupted lines. P, A, M, T, pulmonary, aortic, mitral and tricuspid orifices. Scale in cm.

saline. After 72–96 hr. they were removed and dissected according to a uniform plan to be described below. By controlled daily weighings the loss in weight due to storage in 10 % formol saline for 3–4 days was found not to exceed 2 %, and this was ignored.

*Dissection of the heart.* Throughout the process of dissection the specimen under investigation was kept moist to avoid the loss of weight caused by drying. The steps of dissection are described below and illustrated in Fig. 1.

(1) A narrow strip of pericardium with the underlying vessels was removed from the interventricular grooves on the anterior and inferior surfaces of the heart; the myocardium was damaged as little as possible. This manoeuvre served to fix the peripheral attachment of the septum and the division between the right and left ventricles, as far as the position of the vessels could be relied on to do so. In practice the interventricular branches of the two coronary arteries were easily defined and marked the division between the ventricles accurately, in most cases. Occasionally there was some difficulty on the inferior surface of the heart, due to irregular distribution of the right coronary branch. No attempt was made to remove other

branches of the coronary vessels, and the pericardium was left intact except where it overlay deposits of fat (see para. 4).

(2) By sharp dissection all fat, vessels and the attached atrial walls were removed from the upper surface of each ventricle and from the septum. With experience it was easy to expose with a few strokes of a sharp scalpel the rounded shoulder of ventricular muscle which presents in the atrio-ventricular groove.

(3) The aorta and pulmonary trunk were removed with scissors at their roots; the aorta proximal to the origin of the coronary arteries and the pulmonary trunk at the limit of the infundibular myocardium, a level easily seen with the naked eye.

(4) Any deposits of subpericardial fat were removed with their pericardial covering.

The remaining specimen was now separated into right ventricular, septal, and left ventricular portions by the following incisions. The position of these incisions on the atrial surface of the ventricular mass is shown in Fig. 1.

(5) With a fine pair of scissors, one blade of which was inserted into the tricuspid opening, the infundibulum of the right ventricle was separated from the aorta. The incision passed usually between the anterior and septal cusps of the tricuspid valve, divided the tendon of the infundibulum (conus tendon), and was prolonged a short way down the anterior interventricular sulcus. The muscle of the infundibulum always separated from the aorta as a complete muscular cylinder which extended for a short distance below the level of the attachment of the cusps of the pulmonary valve.

(6) An incision was made from the inferior interventricular groove into the cavity of the right ventricle in such a way as completely to separate the free wall from the septum. The position of this incision was to some extent controlled by the necessity to insert the blade into the groove, but it was possible to incise too deeply into the septum. Consistency is acquired with experience, and the personal error involved is certainly less than the error in attempting to separate the left ventricular free wall from the septum in a consistent way (see para. 8).

(7) A similar incision made from the anterior interventricular groove into the right ventricle divided the last attachment of the right ventricular free wall to the rest of the heart. With rare exceptions the extremity of the rounded apex of the heart was formed by the left ventricle.

(8) From the anterior and inferior interventricular grooves incisions were made into the left ventricle so as to separate its free wall from the septum. The incisions avoided the aorta, the intracardiac portion of which remained with the septal fragment. It was very difficult to make these incisions in a consistent fashion, but as will be shown, the division of the septal fragment from the free wall of the left ventricle can be ignored in analysing the weight ratio of the two ventricles, if they are dissected according to the present plan.

(9) From each of the three fragments the cusps of the mitral and tricuspid valves with their chordae tendineae were dissected away and the semilunar cusps of the aortic and pulmonary valves also removed. The removal of the anterior cusp of the mitral valve naturally opened the aorta widely, and access to the pulmonary valve was obtained by dividing the muscular cylinder of the infundibulum along a convenient line. The roots of the two great arteries were further trimmed if necessary.



*Weighing.* Each fragment was shaken free of moisture by hand and weighed on an ordinary laboratory balance to the nearest 0.1 g. The abbreviations *R*, *L* and *S* will be freely used to represent the weights in g. of the three fragments dissected in the way described, and *T* the total ventricular weight, i.e.  $R + S + L$ .

#### ANALYSIS OF OBSERVATIONS

The difficulty of interpreting the mutual relationships of the three quantities *R*, *S* and *L* naturally resolves itself into a decision about the quantity *S*. The division of the ventricular mass may be regarded as taking place in two stages: the division of the right ventricular free wall (*R*) from the remainder ( $S + L$ ), and the division of the latter into a septal fragment (*S*) and the left ventricular free wall (*L*). The present study is closely concerned with the foetal heart which, in comparison with the adult heart, shows a marked right ventricular 'hypertrophy', diminishing after birth at a rate to be demonstrated. It is therefore evident that the ratio  $R/T$ , which is a quantitative reflexion of the first stage of the division, will provide some clue to this altering relationship. It remains to be shown whether all, some, or none of the septal fragment should be considered as forming part of the right ventricle, a consideration which might modify the impression given by the  $R/T$  proportion alone.

Table 1. *Mean septal proportion with standard errors*

	Mean $S/(S + L)$ (%)	S.E.M.
Birth	32.6	0.54
Neonatal	32.7	1.29
1 month old	32.4	0.79
2 months old	32.0	1.03
3 months old	31.1	0.56
4 months old	31.9	1.04
5th and 6th months	32.4	0.65
7th-12th months	31.9	0.55
2nd year	30.8	1.44
3rd year	31.4	1.17
24 specimens from 4 to 16 years	31.4	0.63
20 specimens from premature infants at birth	32.3	1.23

In the present study the proportion  $S/(S + L)$  expresses quantitatively the second stage of the division. This proportion has been calculated to the nearest whole figure of percentage for each specimen, and the mean observation with its standard error computed for each age group. The results of this analysis appear in Table 1. No figure in this table differs significantly from any other. The inference is clear: in this series of observations the septum (more correctly the septal fragment as divided) follows the free wall of the left ventricle in its increase in weight slavishly, and is not influenced by the behaviour of the right ventricular free wall. In other words, the division of the septal fragment from the left ventricular free wall may safely be ignored and the change in relationship between right and left ventricles measured by the ratio  $R/T$  alone.

It must be clearly understood that the conclusions in the last paragraph do not mean that the septum by its contraction does not assist the right ventricle as well

as the left; but simply that the inevitably crude analysis of the structure of the heart which arises from arbitrary cuts dividing its parts cannot detect the functional relationship of the septum and right ventricle. This must be particularly borne in mind in considering the foetal heart, where the two ventricles are approximately equal in bulk.

As a result of these observations it was decided to analyse in detail the changes in weight of the right ventricular free wall (*R*), the remainder of the ventricular mass (*S+L*), and the total ventricular weight (*T*), with the sole significant relationship *R/T* %. These accordingly are the quantities reported in the following tables and graphs. The proportion *R/T* was calculated to the nearest unit of percentage for each heart.

# RESULTS

The results are reported in Tables 2 and 3, the first dealing with the absolute weights and the second with the proportion *R/T* %. In each table the results are arranged in the age groups already described, and opposite each age appear four observations for each quantity measured; the mean, its standard error (S.E.M.), the range of observations and the standard deviation (S.D.).

Table 2. *Postnatal development of the ventricles: weights of the total ventricular mass and of the parts into which it is divided, in g.*

Age	<i>R</i>					<i>S+L</i>				<i>T</i>			
	No.	Mean	S.E.M.	Range	S.D.	Mean	S.E.M.	Range	S.D.	Mean	S.E.M.	Range	S.D.
Birth	36	6.0	0.26	3.4-8.8	1.6	7.5	0.25	5.2-11.1	1.5	13.5	0.47	8.9-19.4	2.8
Neonatal	10	5.1	0.28	3.7-6.8	0.9	8.3	0.41	6.0-10.1	1.3	13.4	0.64	10.1-15.9	2.0
1 month old	13	4.9	0.21	3.6-6.4	0.7	11.1	0.67	7.1-12.8	2.5	16.0	0.87	10.7-20.7	3.1
2 months old	15	4.7	0.24	3.4-6.8	0.9	11.2	0.56	8.4-16.2	2.2	15.9	0.86	11.8-23.0	3.3
3 months old	14	5.2	0.34	3.5-7.6	1.3	12.8	0.83	8.7-18.6	3.1	18.0	1.13	12.8-26.2	4.2
4 months old	14	5.0	0.28	3.4-6.7	1.0	12.6	0.74	8.4-18.3	2.8	17.6	0.92	11.8-24.8	3.6
5th and 6th months	17	5.5	0.33	3.6-8.6	1.3	13.6	0.98	7.9-26.0	4.1	19.1	1.29	11.5-34.6	5.3
7th-12th months	21	5.6	0.34	3.6-8.8	1.6	14.6	1.05	8.3-26.5	4.8	20.2	1.38	12.1-34.9	6.3
2nd year	22	7.9	0.61	4.6-12.0	2.9	21.7	1.05	12.3-30.8	4.9	29.8	1.39	16.9-41.1	6.5
3rd year	10	10.7	0.78	6.9-14.6	2.5	26.6	1.17	19.2-32.3	3.7	38.3	1.81	31.3-47.6	5.7

Table 3. *Postnatal development of the ventricles: weight ratio *R/T* %*

Age	No.	Mean	S.E.M.	Range	S.D.
Birth	36	44.1	0.61	36-53	3.7
Neonatal	10	38.3	1.18	33-43	3.7
1 month old	13	31.0	1.06	25-39	3.8
2 months old	15	29.7	0.64	25-34	2.5
3 months old	14	28.9	0.69	25-35	2.6
4 months old	14	28.4	0.67	26-34	2.5
5th and 6th months	17	28.9	0.75	25-32	3.1
7th-12th months	21	28.1	0.56	24-31	2.6
2nd year	22	27.4	0.39	24-31	1.8
3rd year	10	27.9	1.00	22-31	3.2
4th-16th years	24	27.2	0.44	24-33	2.2

The justification for excluding six hearts from the normal series can now be stated. Each will be compared with the other specimens in the same age group.

(1) No. 223, neonatal: *T* 21.3 g., *R/T* 48 %. Table 2 shows that the largest specimen at that age weighed 15.9 g., and Table 3 that the mean *R/T* value was 38.3 % and the highest 43 %.

(2) No. 59, 1 month old:  $T$  23.8 g.,  $R/T$  46 % (cf. Tables 2 and 3, greatest  $T$  20.7 g., highest  $R/T$  39 %).

(3) No. 213, 3 months old:  $T$  36.7 g.,  $R/T$  48 % (cf. Tables 2 and 3, greatest  $T$  26.2 g., highest  $R/T$  35 %).

These three specimens demonstrated considerable right ventricular hypertrophy which was not explained by any congenital or acquired cardiac defect that could be determined. It may be surmised that these infants suffered from some form of pulmonary hypertension of early onset.

(4) No. 13, 4 months old:  $T$  30.4 g.,  $R$  10.2 g.,  $R/T$  34 % (cf. Tables 2 and 3, greatest  $T$  24.8 g., greatest  $R$  6.7 g.). This appears to have been a case of generalized ventricular hypertrophy, also unexplained.

(5) No. 26, 14 months old:  $R/T$  36 % (cf. Table 3, highest  $R/T$  value in the second year 31 %).

(6) No. 127, 36 months old:  $R/T$  32 % (cf. Tables 3, highest  $R/T$  value in the third year 31 %).

In cases (5) and (6) the cause of death was generalized pulmonary tuberculosis, and the effect of this in producing right ventricular hypertrophy is clear. No other case of pulmonary tuberculosis was collected.

In addition, four cases were judged as derived from infants prematurely born on evidence of ventricular weight alone. Three of these were each 3 months old and showed  $T$  values of 7.8, 8.3 and 8.8 g. respectively; these weights may be compared with the mean of 18 g. for the other members of this age group and a range of observations extending from 12.8 to 26.2 g. (Table 2). The fourth was from an infant 5 months old and had a  $T$  value of 9.5 g. The range in the 5th and 6th month age group was 11.5–34.6 g. The exclusion of this last case is not as easy to justify as in the case of the other three, but the range of observations is so wide that the exclusion of this one case does not alter the means or deviations very much.

#### INTERPRETATION OF THE RESULTS

In the following statements about the changes in the mean figures with advancing age, no change has been considered significant unless shown to be so by the 't' test with a probability limit of 0.05, and most of the changes on which stress will be laid exceed the probability limit of 0.02 or 0.01 by the 't' test. A rough check on the validity of the statements made is available in the standard errors of the means.

*T values.* The total ventricular mass appears, from the figures in Table 2, to increase in a rather irregular step-like fashion. There is little doubt that the irregularities would be smoothed out if the numbers in each group were very much larger. The rate of increase represented by the gain of 2.5 g. on 13.5 g. (18 %) in the first month is greater than at any subsequent period. The same statement can be made about the increase of 4.5 g. (33 %) in the first 3 months. After this point the errors of the means do not seem to justify the drawing of any other than a straight line through the points on the graph (see Fig. 2); in other words, from the age of 3 months onwards the rate of increase in the total ventricular weight is probably reasonably steady at about 0.7 g. per month.



*S+L values.* The growth of the septum and left ventricle provides an even greater contrast between the rate of growth in the first month and that shown in later months. A birth weight of 7.5 g. increases to 11.1 g. (48 % increase) in the first month. This spurt rapidly ceases, and after the age of 3 months the figures seem to indicate a steady rate of increase of about 0.5 g. per month.

*R values.* There is a sharp reduction of the weight of the right ventricular fragment in the first month, from 6.0 to 4.9 g., amounting to a loss of about 20 %. From then until the age of 4 months there is no change in the mean weight of the right ventricular fragment which is statistically significant. The lowest figure in the column,

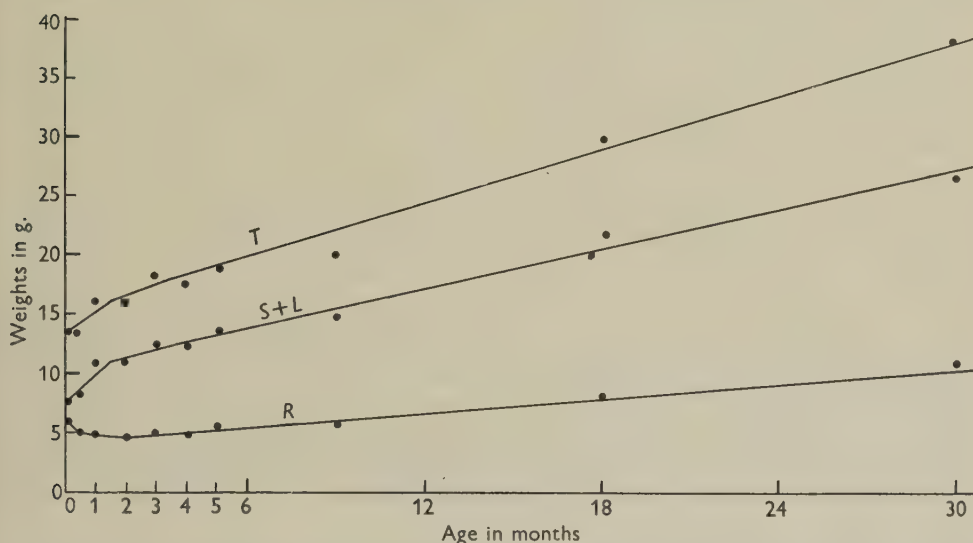


Fig. 2. The ventricular development after birth. *T* is the total ventricular mass; *R* is the free wall of the right ventricle; *S+L* is the septum and free wall of the left ventricle, i.e.  $T - R$ .

4.7 g. at 2 months, cannot be distinguished from any which follow until the 5th and 6th months age period is reached, showing a mean weight of 5.5 g. The birth mean of 6.0 g. is not reached until the second half of the first post-natal year, and is first exceeded by the figure for the second year. The last three figures in the column suggest an average rate of growth of just over 0.2 g. per month.

Some doubts may be felt about the validity of the age changes expressed in the last three paragraphs, in view of the arbitrary selection of the postnatal cases in the effort to avoid premature subjects. Thus if too many were excluded the appearance of rapid growth of *S+L* and also of *T* in the first 3 months might be spurious. On the other hand, if premature specimens were included, then the atrophy of the right ventricular free wall might have been exaggerated. However, as the two parts into which the ventricular mass was divided move in opposite directions, the one growing rapidly while the other atrophies, the two possible objections to some extent cancel each other. Thus if prematures were included, the rapid increase of *S+L* would be difficult to account for; and if too many specimens were excluded in error, then the

true shrinkage of the right ventricular fragment would be even more striking than it already appears.

The changes in  $R$ ,  $S+L$  and  $T$  values with age are graphically shown in Fig. 2.

*R/T values.* The variations of the observations about the mean figures is much less in the case of the proportions reported in Table 3 than it is in the case of the absolute weights in Table 2. The standard deviations in Table 2 average 20 % of the mean figures, while the corresponding comparison in Table 3 shows s.d. figures usually about 10 % of the mean. In other words, the proportionate development of the two ventricles is less variable than their absolute weight; this conclusion is not, perhaps, very surprising, but it does indicate that the methods used do reasonably measure the mutual relationship of the two ventricles.

Starting with the birth figure of 44 %, the  $R/T$  value drops sharply in the first month to 31 %. This rapid change of relationship is brought about by the weight changes in opposite directions which have already been demonstrated—the hypertrophy of the septal fragment and the left ventricular free wall, together with the atrophy of the right ventricular free wall. This rate of change in the relationship of the two ventricles is not approached in the subsequent months; in fact more than three-quarters of the total change seen in Table 3 occurs in the first month.

The mean figure of 31.0 % at 1 month cannot be distinguished from those that follow until the figure of 28.4 % at 4 months. After this point it is possible to demonstrate change only by comparing the forty-five cases in the period 3–6 months with the seventy-seven cases from all the subsequent age periods. The mean figure for the earlier group is 28.7 % and this is found to differ significantly from the mean of 27.6 % from the later cases. Stability is reached with the mean figure of 28.1 % at 7–12 months, because this cannot be distinguished from the mean values for the later periods.

It is possible that an increase in the numbers of hearts examined would show up further slight change. With the s.d. figure remaining about 2.5 %, more than sixty observations would be necessary in each age group to detect a significant change of 1 % in the mean figure, and about 250 at each age to demonstrate a change of 0.5 %. From a practical point of view there seems to be no change in the *relative* development of the two ventricles after the first six months of life.

To recapitulate in round figures, the  $R/T$  proportion changes from 44 % at birth to a steady level of about 28 % in 6 months. Of the total drop of 16 %, 13 % is achieved in the first month, a further 2 % between 1 and 3 months and the remaining 1 % between 3 and 6 months of age. These changes are graphically shown in Fig. 3. Evidence will be presented later that the final level reached is the same as is found in normal adult hearts.

*Premature cases.* As mentioned above, thirty-five hearts from infants considered to have been born prematurely were also examined. The twenty which were from stillbirths or from infants dying in the first 3 days gave the following  $R/T$  figures, which compare reasonably well with the figures under 'Birth' in Table 3:

$R/T$ %	Mean	S.E.M.	Range	S.D.
Premature infants at birth	42.0	0.99	34–48	4.4

Those which survived beyond 3 days were too few to analyse collectively and are now reported individually:

Premature infants	No.	<i>R/T</i> %
1 week old	6	37, 40, 42, 44, 44, 45
2 weeks old	1	45
3 weeks old	2	30, 34
1 month old	1	27
3 months old	3	26, 30, 36
4 months old	2	29, 33
5 months old	1	27

These figures, for what they are worth, show that the influence of extra-uterine life on the heart is the same for these premature infants as for those born at full term; particularly striking are the values at 3 weeks and 1 month old.

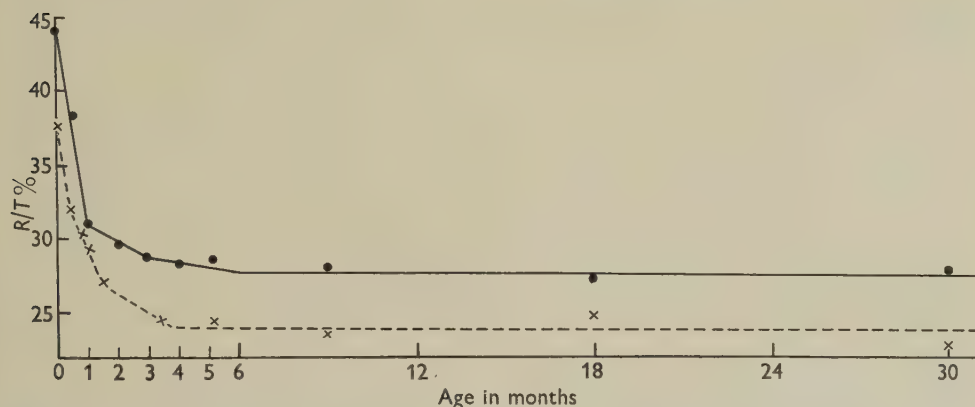


Fig. 3. The change in *R/L* ventricular ratio with age. —, Figures from present study; - - x - -, figures from W. Müller (1883). *R* is the weight of the right ventricular free wall, and *T* the total ventricular mass.

#### THE WORK OF W. MÜLLER (1883)

Müller dissected a very large number of hearts in the fresh state. The description he gave of his method of division of the ventricular mass is not altogether clear. He allotted the septal fragment to right and left ventricles according to the proportion 0.3021 to the right ventricle and 0.6979 to the left ventricle. These proportions he derived from an argument, which would be unacceptable to-day, about adult hearts showing right or left ventricular hypertrophy. Moreover, he used this proportional allotment of the septal fragment for all the foetal and infantile specimens as well as the adult hearts; this cannot be justified as a foetal heart is by no means a miniature adult heart. Müller accepted all cases, whether prematurely born or not, into his series. This will be obvious from the following mean weights of the total ventricular mass, as Müller reported them for the several age groups: 13 stillbirths (weighing between 2.5 and 3.0 kg.) 15.1 g.; 33 dying in the first week 12.0 g.; 15 dying in the third week 13.3 g.; 28 dying in the second month 12.4 g. The final difficulty about accepting Müller's work is that he wrote before statistical analysis of means and variance was widely understood.



Despite these objections it is worth quoting Müller's conclusions as they affect the present problem because, in contrast to the figures in his tables, they have received little attention. Müller believed that at full term the work of the two ventricles was about equally divided between them. Considering the postnatal change, he continued:\*

P. 210: . . . comparison of the figures for the first month of extra-uterine life with those of later foetal life shows that during the first month after birth the right ventricle loses weight, and the left gains weight; loss and gain occur much more rapidly in the first and second weeks than in the third and fourth.

The cause of this change must be that birth relieves the right ventricle of some of its work, while the left ventricle is increasingly loaded. . . .

P. 212: After the pulmonary and systemic circulations are completely separated, which appears to happen at the beginning of the second month of life, an unequal increase in weight of the two ventricles takes place, which continues throughout the remainder of the first year. In this way the permanent proportion between the weights of the two ventricles is first established at a time when independent nourishment has started, and the infant learns to walk upright.

However, consideration of Müller's findings by modern statistical techniques shows that he was not justified in assuming any change in right/left ventricular relationship after the fourth month, so that he was not entitled to his conclusion in the second half of the above quotation. In addition, the fact that he included numerous premature cases in his postnatal series makes his statement about right ventricular atrophy less well-founded than it otherwise would have been. However, it will have been observed that this statement has been confirmed in the present study.

*Re-analysis of Müller's observations.* By tracing the individual specimens through the various sections of Müller's detailed report, it is possible to find out the weight and length of all of the infants from which his specimens were derived. Many of the infants were seen to have been prematurely born when these data were examined. In order to provide a rough comparison between Müller's work and the present study, it was decided to exclude from Müller's series the more obviously premature cases. To this end a generous lower limit of 2.0 kg. and 45 cm. was used for infants up to 2 months and the same weight but 50 cm. for the period 3-6 months. The object of setting such a low limit as 2.0 kg. was to make every allowance for normal loss of weight as well as the effect of malnutrition on infants whose birth weight might have been 2.5 kg. or more. As will be seen from the results, however, there are grounds for thinking that these limits were not severe enough and that many premature cases remain included in the series. Forty of 192 postnatal cases in the first 6 months were excluded from Müller's original series on the grounds stated.

The next difficulty arises from differences in the two methods of dissection of the ventricular mass. This can best be illustrated by showing the mean proportions of the three fragments as separated by Müller and comparing them with the proportions seen in the present study. The following are the mean percentage distributions, to the nearest whole number, of *R*, *S* and *L* at two representative age periods. As Müller's classification into age categories is not quite the same as that used in the

\* Author's translation of the original German.

present study, the author's 'Birth' category is compared with Müller's stillborn cases weighing 2.5-3.0 kg.:

	<i>R/T</i> %	S.D.	<i>S/T</i> %	<i>L/T</i> %	<i>S/S+L</i> %
Müller results, stillborn 2.5-3.0 kg.	38	3.5	30	32	48
Present study, 'birth'	44	3.7	18	38	33
Müller's results, 7th-12th months	24	3.4	33	43	43
Present study, 7th-12th months	28	2.4	23	49	32

It is apparent from these figures that in Müller's method the septal fragment is much larger than in the present study. The reason for this can be understood from the description Müller gives of his method. It is larger at the expense of the free walls of both right and left ventricles. The effect of including part of what is considered in the present study to be right ventricular free wall with the septal fragment can be seen in the last column. This shows that by Müller's method there is not the constancy in proportion between the septal fragment and the free wall of the left ventricle which was found in the present study (see Table 1). Direct comparison of the weights of the fragments was therefore out of the question. However, on the assumption that Müller was consistent in his method, it seemed reasonable to inquire whether his figures, analysed according to the author's method, showed similar growth changes or not. Accordingly, *R*, *R/T* % and *T* values were tabulated for all the cases remaining after exclusion of the premature cases. These figures appear in Table 4.

Table 4. *Postnatal development of the ventricles: Müller's findings corrected by the omission of premature subjects. Weights in g.*

Age	<i>R</i>					<i>R/T</i> %				<i>T</i>			
	No.	Mean	S.E.M.	Range	S.D.	Mean	S.E.M.	Range	S.D.	Mean	S.E.M.	Range	S.D.
Stillborn													
2.5-3.0 kg.	13	5.7	0.24	4.1-7.7	0.8	37.7	1.02	31-46	3.6	15.1	0.35	13.4-17.4	1.2
3.0 kg. and more]	22	7.5	0.46	5.4-11.8	2.1	41.4	1.01	34-51	4.7	18.1	0.77	13.1-24.0	3.6
Live-born													
1st week	17	5.9	0.38	3.5-8.4	1.6	36.9	1.40	21-46	5.8	16.1	0.95	10.3-22.7	3.9
2nd week	21	4.5	0.19	2.9-6.3	0.8	32.0	0.65	27-38	3.0	14.0	0.51	10.3-18.3	2.3
3rd week	13	4.2	0.29	2.9-6.0	1.4	30.6	0.58	28-34	2.0	13.8	0.95	9.9-19.7	3.4
4th week	11	4.0	0.22	2.7-5.4	0.6	29.5	0.78	24-32	2.6	13.5	1.02	9.4-20.8	3.4
2nd month	21	3.6	0.13	2.7-7.5	0.6	26.9	0.67	24-33	3.1	13.4	0.63	9.4-19.6	2.9
3rd month	27	4.0	0.22	2.7-7.5	1.2	25.7	0.78	19-40	4.1	15.8	0.83	10.5-28.4	4.1
4th month	19	4.3	0.33	2.6-7.7	1.4	24.6	0.81	18-32	3.5	17.6	1.21	10.2-26.8	5.3
5th and 6th months	23	4.9	0.31	2.7-8.5	1.5	24.6	0.69	20-33	3.3	19.9	0.92	11.1-26.7	4.4
7th-12th months	64	5.8	0.18	2.7-10.7	1.4	23.7	0.43	16-39	3.4	24.5	0.73	11.5-38.2	5.8
2nd year	41	8.3	0.50	4.2-22.0	3.2	25.0	0.72	19-38	4.6	32.6	1.25	19.8-57.0	8.0
3rd year													
Male	13	10.6	0.71	7.7-15.1	2.6	22.7	0.66	19-36	3.6	46.5	2.66	32.0-69.6	9.6
Female	16	9.0	0.58	6.1-14.0	2.3					40.2	1.74	33.2-53.0	7.0

Comparison of the *T* values with those in Table 2 shows some puzzling inconsistencies. Some of these may be due to the selection of Müller's postnatal cases being faulty; others are not open to the same explanation. In particular, there is no suggestion of the rapid growth in the first 3 months which was so obvious in the present study.

Turning to the *R* values, a change similar to that found in the present study is seen. Comparing the figure for the second week (*T* 14.0 g., *R* 4.5 g.) with those from the second month (*T* 13.4 g., *R* 3.6 g.) there is a significant diminution in the weight of

the right ventricular free wall. This does not regain its birth weight until the second half of the first year and is first exceeded by the figures for the second year. The atrophy is of the same order as in the present study, that is a loss of about 20 % to its lowest point at 2 months.

The  $R/T$  % figures show, by comparison of the standard deviations, that Müller was about as consistent as the author in dividing up the ventricular mass. Although the figures are throughout lower, for the reasons already explained, there is the same rapid readjustment of the ventricular ratio. There is a decrease of about 10 % in the first month from the initial level of approximately 40 %. After this the rate of changes diminishes considerably, and when the figure of 24.5 % for the 4th month is reached, no further change which is statistically significant can be detected. The  $R/T$  proportion for the second year age group is higher than the figures before or after it. Müller remarked on this feature of the series and gave his own opinion that it was a chance finding due to the inclusion of cases in which the disease process causing death had also caused right ventricular hypertrophy. But for this figure it might have been possible to show some change after the fourth month. These changes are illustrated graphically in Fig. 3, and the similarity in the proportionate growth change shown by the two studies is obvious.

Broadly, then, fresh examination of Müller's work by improved statistical technique re-enforces the conclusions reached in the present study. The readjustment between the bulk of the right and left ventricles is rapid, the greater part occurring in the first month of life. Finality in this relationship appears to be reached within the first 6 months. There is a similar reduction in the mass of the right ventricular free wall in the first month or two, which is not restored for nearly a year.

## DISCUSSION

### (1) *The assessment of the ventricular ratio*

The method of analysis used in the present study, in which the septum is considered as forming a unit with the free wall of the left ventricle, has already been justified. The usefulness of the method is that it allows easy comparison of one heart with another, or of one group of hearts with another. It does not provide a measure of the precise proportion in which the weight of the ventricular myocardium should be divided between right and left sides and does not, in consequence, permit a precise estimate of the relative anatomical or physiological status of the two ventricles.

Those who have made gravimetric studies of the heart have usually attempted to establish some theoretical rule which would enable them to state a precise ventricular weight ratio, and have commonly proceeded without hesitation on the assumption that such a ratio accurately reflects the functional relationship of the two ventricles. Müller's methods have already been considered. Lewis (1914), in a classical study of the effect of ventricular hypertrophy on the electrocardiogram, felt dissatisfied with Müller's account and adopted a method of division which separated each ventricle from the septum as an intact chamber. He did this because he thought that the trabeculated surfaces of the septum belonged functionally to the side to which they presented. Hermann & Wilson (1922), in a similar study, stated that they were unable to follow the details of Lewis's method and preferred to partition the septum



by dividing it along a white line (seen in successive sections of the septum) which they considered indicated the functional division of the septum. Fulton, Hutchinson & Morgan Jones (1952) rejected the methods of their predecessors as difficult to follow and of doubtful theoretical validity. Instead they divided the ventricular mass by a method closely resembling that followed in the present study. Having established a range of observations for normal adult hearts, they found that in right ventricular hypertrophy the septal fragment hardly ever exceeded its normal range of weight. On the other hand, it exceeded its normal limits in almost all cases of left ventricular hypertrophy. As a result they decided, as in the present study, to consider the septum and free wall of the left ventricle as one part of the heart, to be compared with the free wall of the right ventricle in estimating ventricular hypertrophy. They made no attempt to establish absolute weights for the two ventricles.

It is clear that there is no agreed method by which the weight of each ventricle can be precisely stated. It is doubtful whether any method would survive critical scrutiny. The procedure adopted by Fulton *et al.*, and in the present study, is simple and less liable to misinterpretation than those quoted, and is quite capable of being used to compare one heart with another, or with a normal standard.

Even if some acceptable method of allotting the septal fragment were proposed, theoretical considerations would make it hazardous to base functional conclusions on the anatomical weight ratio so established. The two ventricles are not of the same shape, and it is not permissible to assume that they act as equally efficient pumping units. This can be illustrated by the findings of Fulton *et al.* For forty-three normal hearts from adults under the age of 65 they reported the following weights of the divided ventricular mass (in g.):

	<i>R</i>	<i>S</i>	<i>L</i>	<i>T</i>
Mean	46	39	86	171
Maximum	68	61	123	252
Minimum	23	17	48	88

Taking the mean figures, the weight of the right ventricular free wall is seen to amount to 37 % of the weight of the septum and free wall of the left ventricle. This means that the weight of myocardium active on the right side averages at least one-third of the weight of the left ventricle, if not more. But direct studies of human ventricular pressures have shown that the right ventricle does a fourth part or less of the work of the left, in the normal heart. The discrepancy is obvious, and it is clear that the relative functional status of the two ventricles cannot be accurately assessed by dissection and weighing of the ventricular myocardium.

If these arguments are accepted it follows that any statement of relative ventricular status, based on dissection of the heart, which is precise will be suspect. On the other hand, a method of dissection, such as that used in the present study, consistently applied provides a satisfactory means of comparing the relative ventricular status of one heart with another, or with an established normal range of variation.

## (2) *The foetal ventricular ratio*

Many authors have followed Spigelius and Harvey's lead and stated that the two foetal ventricles are equal in thickness and in other respects, and have inferred that they are functionally balanced.

Two other views, mutually contradictory, have also been put forward. Scammon (1923) and Brock (1932) both use Müller as authority for the statement that the ratio left/right ventricle at birth is  $5/4$ . Inspection of these authors shows that they accepted Müller's method and analysis uncritically and also that they treated his figures from the first week as representing birth conditions. In view of the rapid postnatal change in the ventricular ratio shown in the present investigation this was not, perhaps, a justifiable inference. The opposite view is expressed by Patten (1930), who believed on the basis of examination of over fifty hearts from stillborn infants that the right ventricle outweighed the left by  $8/7$ . Patten has not published the method used in the investigation which led to this conclusion.

The objections to precise statements such as those quoted have already been stated. While precision cannot be expected, an approximation is not ruled out. It is obvious on inspection that the two ventricles in the foetus are more nearly equal in status than they are in the adult. In the present study the free wall of the right ventricle amounted, at the time of birth, to 44 % of the total ventricular weight on the average, and in several cases exceeded 50 %. The septal fragment in these hearts averaged 18 % of the total. If, for argument's sake, the septum were considered to be neutral in status then half its weight would go to each side. On this assumption the ventricular weight would be divided in the ratio right 53, left 47. Judging simply from appearances during dissection of these hearts, the right ventricle often appears more bulky than the left. These figures show that in late foetal life the weight of the ventricular myocardium is divided about equally between the two sides, although in some cases the musculature of the right ventricle weighs more than that of the left. There is some slight support for the idea that the right ventricle is, on the average, the heavier. The objections to proceeding, without further evidence, from these statements to more precise functional conclusions have already been discussed.

### (3) *The postnatal transformation of the foetal ventricles*

As in the case of the foetal ventricular ratio several differing statements about postnatal ventricular changes can be found in the literature. Lewis (1914) thought that Müller had '...conclusively shown right sided preponderance in the new-born child and its gradual decline towards the third month...'. It will be seen that he did not follow Müller's own conclusions. Scammon (1923), also relying on Müller, stated that the ratio left/right ventricle was  $5/4$  at birth,  $2/1$  at the age of 2 years and even more at puberty. Patten (1930) thought that Müller (as quoted by Gross, 1921) had shown that the '...full adult degree of preponderance is reached around the seventh year'. Gross's quotation of Müller's work is unfortunately misleading. Brock (1932) thought Müller's figures showed changes in right/left ventricular ratio with age until 6-10 years. His quotation of Müller's work is incorrect in several particulars. J. A. Keen (1942) published planimetric observations on cross-sections of foetal and infantile ventricles. This method is open to several criticisms and too few hearts were examined to allow firm conclusions. In their book *The Foetal Circulation*, Barclay, Franklin & Prichard (1944) devote little attention to postnatal ventricular change. There is no mention of the course of events in the lamb, the subject of the classical radiological studies. In considering the human heart they cite electrocardiographic evidence and also quote Patten on the foetal ventricular ratio,

but they give no precise estimate of the rate of the postnatal change. A typical opinion from electrocardiographic studies can be seen in Alimurung, Joseph, Nadas & Massel (1951). These authors studied 521 children and observed continuing changes in the normal appearances up to the age of 13 years. They considered these changes mainly due to changes in the relative sizes of the ventricles, quoting Brock (1932) and Patten (1930) as authorities.

The quoted statements indicate a wide variety of opinion on the rate of the postnatal transformation of the ventricular balance. The present investigation, which is supported by a re-analysis of Müller's figures, shows that the period of most rapid change in the ventricular ratio is in the first month of life. After this a more gradual change takes place, until at 6 months the left ventricle has reached a degree of preponderance which no longer changes with increasing age (Table 3 and Fig. 2). The final steady level of ventricular relationship during childhood can fortunately be compared with the ratio characteristic of the normal adult heart through the study of Fulton *et al.* already mentioned. The similarity of the method of dissection used by these authors and that in the present study can be illustrated by the following figures:

	<i>R/T</i> %	<i>S/T</i> %	<i>L/T</i> %
Fulton <i>et al.</i> (1952)			
43 normal hearts from adult subjects	26.9	22.8	50.3
Present study			
24 normal hearts from subjects aged 4-16 years	27.2	22.9	49.9

It is obvious that the *R/T* figures from the two studies are almost identical. It is a reasonable conclusion that the ventricular weight ratio reached after 6 months of life remains characteristic of the rest of childhood, adolescence, and normal adult life. In other words, growth of the ventricular mass after 6 months of age occurs in such a way that the increments to each side are proportionate to their bulk. The slow electrocardiographic changes in childhood must be caused by factors other than a change in the ratio of ventricular bulk, after the first 6 months of life.

#### (4) *Postnatal atrophy of the right ventricle*

The loss of weight of the right ventricular free wall after birth is interesting and significant. Müller, as has been shown above, deserves the credit for first observing this phenomenon. His explanation of the atrophy was that immediately after birth the work of the right ventricle was diminished. This conclusion must be endorsed as the only convincing explanation of the fact. The whole process shows that the healthy myocardium responds to a diminution of the work it is required to do in the same way as skeletal muscle.

Boellaard (1952) has published a study of the microscopic diameter of cardiac muscle fibres taken from right and left ventricles in the postnatal period. His findings may be summarized as follows:

#### *Diameter of ventricular muscle fibres ( $\mu$ ), Boellaard (1952)*

Age	Right ventricle			Left ventricle		
	No. of specimens	Range of observations	Mean diameter	No. of specimens	Range of observations	Mean diameter
Newborn to 40 hr.	10	4.5-6.9	5.6	3	3.7, 4.7, 5.0	4.5
6 weeks to 10 months	9	4.6-5.4	5.1	5	5.3-6.6	6.0



The reduction in diameter of the right ventricular fibres compares well with the loss in weight shown in the present study, according to the following argument. Assuming that the length of the fibres remains unchanged, the volume of the fibres will be proportional to the cross-sectional area of the fibres, itself proportional to the square of the radius. Therefore the reduction in volume of the fibres will be of the order of  $(5.1/5.6)^2$ , or from 100 to 84 %. The total reduction in weight of the right ventricular free wall is from 6.0 g. at birth to 4.7 g. at 2 months, i.e. from 100 to 78 %. These figures are very similar. In contrast the fibres of the left ventricle hypertrophy considerably in the corresponding period.

Unfortunately Boellaard believed that Müller had shown a gradual increase in right ventricular weight from birth onwards. As has been noted, he was not alone in this mistaken belief. This forced him to conclude that a rapid growth in length of the right ventricular fibres occurred. In view of the correspondence of loss of weight with reduction in diameter no such assumption is necessary.

Smith (1951) specifically denies any right ventricular atrophy. As he relies on Brock (1932), whose quotation of Müller was unreliable and incomplete, it is not surprising that he was misled.

Patten (1930, 1951, 1953) has in several publications maintained that the event of birth has little immediate effect on the foetal cardiovascular system. He has maintained that flow in the foetal direction continues through the ductus arteriosus and foramen ovale until these passages are anatomically obliterated. In his *Human Embryology* (1953), on p. 671, he writes '... the right ventricle at all times carries its full share of the pumping load. Were this not the case the muscular development of the right ventricle would not be sufficient at the time of birth to meet the load it must carry when the pulmonary circulation becomes functional'. He believed, as has been mentioned, that the change in relative ventricular status was a gradual one, extending over 7 years.

It is clear that if the findings of the present study are accepted, Patten's view of the cardiovascular adjustments at birth cannot be supported. Far from being insufficiently developed to meet the load of the pulmonary circulation, the right ventricle is found to have developed in the later foetal months to a point where it deals with more work than it is required to do after birth. Nor can any support be given to the idea that the changes in the heart are gradual and extend over years; the change in ventricular proportion can better be described as rapid than gradual, as the greater part of the readjustment takes place in the first month of life.

The right ventricular atrophy permits one further inference. After birth the right ventricle is no longer meeting and overcoming the resistance represented by the pressures in the aorta. The reduction in peripheral resistance which accompanies expansion of the lungs is quite sufficient to account for the reduction in right ventricular work. But flow through the ductus arteriosus in the foetal direction must cease. Whether rapid functional closure of the ductus or reversal of flow in the vessel for a period is the normal process in the human infant has yet to be determined. It is interesting to note that Dawes, Mott, Widdicombe & Wyatt (1953) have recently asserted that in the lamb reversal of flow is normal for up to  $1\frac{1}{2}$  hr. after respiration begins. The final answer to this problem in the case of the human infant must await cine-radiographic observations such as proved so successful in the foetal lamb.

SUMMARY

1. Anatomical examination of the heart cannot be expected to provide a precise estimate of the proportion in which the ventricular myocardium should be divided between right and left sides. Even if this were possible, it does not appear that conclusions about the relative amount of work done by the two ventricles can be based on observations of the proportionate ventricular myocardial weight.

2. Despite these difficulties it is possible, by suitable dissection and weighing, to compare the degree to which the left (or right) ventricle preponderates in various hearts. By the method used in the present study, the weight of the free wall of the right ventricle is compared with the total ventricular weight, and this method is shown to provide a satisfactory comparative measure of the relative bulk of the two ventricles.

3. This method of study has been applied to a series of normal hearts from newly born babies, infants and children. At the time of birth the weight of the myocardium of each ventricle is approximately the same, though in some cases the right ventricle weighs more than the left. Change in this proportion occurs rapidly after birth, so that the left ventricle soon comes to preponderate. The greater part of the total change observed occurs in the first month of life. A slower change proceeds subsequently until the age of 6 months, by which time the relative development of the two ventricles has reached proportions which remain characteristic of the rest of infancy, childhood, adolescence and normal adult life.

A re-examination of the results of the investigations of W. Müller (1883) supports the conclusions of the present study. Müller's own conclusions about the postnatal change in ventricular proportions cannot be supported, and those who have quoted results from Müller's work have usually drawn from them conclusions which appear to be even less justified.

4. During the first month of life the rapid adjustment of ventricular weight ratio results from atrophy of the right ventricle (diminution of the weight of the myocardium of its free wall by about 20 %) during a time of rapid left ventricular growth. The loss of weight is not restored until the end of the first year. This observation means that at or very soon after birth circulatory changes occur which radically alter the amounts of work performed by the two ventricles. The rapid change in ventricular weight ratio does not necessarily imply early cessation of flow through the ductus arteriosus in the human infant, although this explanation must be considered. The right ventricular atrophy does, however, mean that the flow in the ductus is either reversed or halted.

The published statements of Patten on the circulatory adjustments at and following birth are reviewed. The idea that the right ventricle gradually stops pumping blood through the ductus during the early postnatal period is not consistent with the observed atrophy of the right ventricle.

I am indebted to Prof. R. Turner and Prof. J. G. Thomson for permission to collect material from the mortuaries under their control. I am grateful to the following pathologists who helped me by setting aside specimens—Drs J. N. Coetsee,

J. C. E. Kaufmann, J. W. Mostert, J. B. Selkon, F. J. Bennett, N. H. Aldridge and P. L. Botha.

I am also grateful to Sgt. A. C. Vlok, of the Police Mortuary, Cape Town, for assistance in the collection and labelling of specimens.

## REFERENCES

- ALIMURUNG, M. M., JOSEPH, L. G., NADAS, A. S. & MASSEL, B. F. (1951). The unipolar precordial and extremity electrocardiogram in normal infants and children. *Circulation*, **4**, 420-429.
- BARCLAY, A. E., FRANKLIN, K. J. & PRICHARD, M. M. L. (1944). *The Foetal Circulation*. Oxford: Blackwell.
- BOELLAARD, J. W. (1952). Über Umbauvorgänge in der rechter Herzkammerwand während der Neugeborenen- und Säuglungsperiode. *Z. Kreisförsch.* **41**, 101-111.
- BROCK, J. (1932). *Biologische Daten für den Kinderarzt*, p. 132. Berlin: J. Springer.
- DAWES, G. S., MOTT, J. C., WIDDICOMBE, J. G. & WYATT, D. G. (1953). Changes in the lungs of the new-born lamb. *J. Physiol.* **121**, 141-162.
- FULTON, R. M., HUTCHINSON, E. C. & MORGAN JONES, A. (1952). Ventricular weight in cardiac hypertrophy. *Brit. Heart J.* **14**, 413-420.
- GROSS, L. (1921). *The Blood Supply to the Heart*, p. 111. London: Henry Frowde, Hodder and Stoughton.
- HARVEY, W. (1628). *Exercitatio anatomica de motu cordis et sanguinis in animalibus*. Francoforti, Sumptibus Gulielmi Fitzeri.
- HERMANN, G. R. & WILSON, F. N. (1922). Ventricular hypertrophy: a comparison of electrocardiographic and post-mortem observations. *Heart*, **9**, 91-147.
- KEEN, J. A. (1942). A note on the closure of the foramen ovale and the post-natal changes of the ventricles in the human heart. *J. Anat., Lond.*, **77**, 104-109.
- LEWIS, T. (1914). Observation on ventricular hypertrophy with especial reference to preponderance of one or other chamber. *Heart*, **5**, 367-403.
- MÜLLER, W. (1883). *Die Massenverhältnisse des menschlichen Herzens*. Hamburg und Leipzig: Leopold Voss.
- PATTEN, B. M. (1930). The changes in the circulation following birth. *Amer. Heart J.* **6**, 192-205.
- PATTEN, B. M. (1951). *Morris' Human Anatomy*, 10th ed. p. 780. Philadelphia: Blakiston.
- PATTEN, B. M. (1953). *Human Embryology*, 2nd ed. p. 671. New York: Blakiston.
- SCAMMON, R. E. (1923). A summary of the anatomy of the infant and child. *Abt's Pediatrics*, p. 393. New York: Saunders.
- SMITH, C. A. (1951). *The Physiology of the Newborn Infant*, 2nd ed. p. 89. Oxford: Blackwell.
- SPIGELIUS, A. (1626). Quoted by Franklin K. J. (1941). A survey of the growth of knowledge about certain parts of the foetal cardio-vascular apparatus, and about the foetal circulation, in man and some other mammals. Part I: Galen to Harvey. *Ann. Sci.* **5**, 57-89.



# THE CAROTID LABYRINTH IN *HYLA AUREA*, WITH A NOTE ON THAT IN *LEIOPELMA HOCHSTETTERI*

By J. B. CARMAN

*Anatomy Department, University of Otago, Dunedin, New Zealand*

## INTRODUCTION

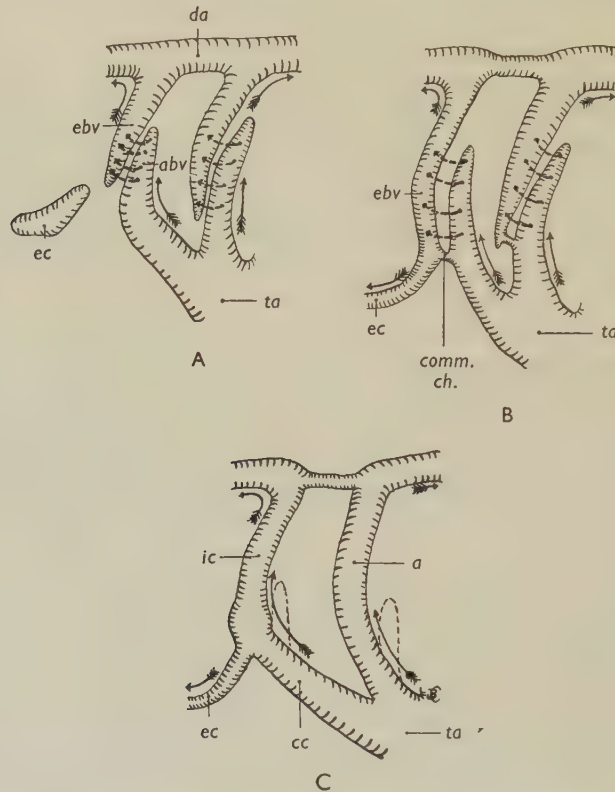
The presence of a specialized structure at the bifurcation of the common carotid artery in the Amphibia has been known for well over 100 years. This structure, which has been variously called the carotid gland, the carotid labyrinth, or the carotid body, is a well-marked swelling, partly cavernous and partly plexiform, at the termination of each common carotid artery; from it arise the external carotid (or lingual) and the internal carotid arteries. The labyrinth occurs in all the common species of urodeles and anurans, but is said to be absent in the Gymnophiona—limbless, burrowing Amphibia.

Since first described by Huschke in 1831, it has been given many functions, such as that of an accessory heart (Hyrtil, 1838; Sabatier, 1873; Boas, 1882, 1883); a gland (Stannius, 1846); and (a view still prevalent) a means of ensuring for the carotids the most richly oxygenated blood (Brücke, 1852; Zimmermann, 1887). Others, comparing it with the carotid body of Amniotes, see in it a possible chemo-receptor: de Boissezon (1939), for instance, says it contains cells very like those in the mammalian carotid body, and recently Chowdhary (1951) has made a similar claim; Pischinger (1934), however, denies this, believing that it contains only ordinary vascular elements. Then again, because Meyer (1927) found depressor fibres in the glossopharyngeal nerve and since this sends a branch to the organ, some have assumed it to be a pressor-receptor: thus, Ask-Upmark (1935) likens it to the mammalian carotid sinus (and perhaps to the extracranial rete mirabile in some lemuroids), and Neil, Ström & Zotterman (1950) also incline to the same opinion, although, apart from one solitary instance (Palme, 1934), sensory endings have, to my knowledge, never been found here. De Boissezon (1939) regards the labyrinth, not as a special pressoreceptor, but rather as a mechanical regulator of the cerebral blood pressure; and Pischinger (1934) has suggested that it actively controls the volume of blood entering the internal carotid, a control necessitated, he believed, by the amphibious habits of the animal.

The internal anatomy of the organ has been studied in detail by relatively few workers (Zimmermann, 1887; Pischinger, 1934; Eloff, 1935; and Ishida, 1954); of these only Ishida has given adequate illustrations of its interior, while the only reconstruction is that by Pischinger, and even this shows only a part. Histologically, some speak of it as muscular, others as fibro-elastic, while de Boissezon calls the tissue of the plexus a 'plasmodium'. Eloff (1935) has described in it cells resembling nerve-cell bodies.

In contrast to the somewhat incomplete and controversial accounts of its structure and functions, the development of the labyrinth has been more thoroughly investigated and, apart from an initial error by Maurer (1888), the results, on the

whole, agree. Marshall (1893), for instance, described the process in *Rana temporaria* as follows: in the tadpole the branchial circulation is typically that of a gill-breather—in each arch an afferent vessel from the truncus lies immediately caudal to the efferent vessel, going to the dorsal aorta, the two being joined at first only by the gill-capillaries (Text-fig. 1A); later (12 mm. stage) they become connected more directly, ventral to the capillary loops, by a small channel (Text-fig. 1B), which so



Text-fig. 1. Schematic drawings illustrating the development of the external carotid and the vascular changes in the first and second branchial arches at metamorphosis (after Marshall and Pischinger). A, 9 mm. stage; B, 12 mm. stage, just before metamorphosis; C, just after metamorphosis. Gill capillary loops are represented by broken arrows, and the direction of blood flow is shown by continuous arrows. For meaning of abbreviations in text-figures and Plate see p. 525.

enlarges at metamorphosis that blood from the heart can now pass directly to the dorsal aorta through the efferent vessel, the gills receiving less and less blood as the pulmonary circulation is established (Text-fig. 1C). Each external carotid anlage arises (9 mm. stage) in the floor of the mouth as a short blind lumen (Text-fig. 1A) whose posterior end soon turns outwards and dorsally towards the ventral end of the efferent vessel of the first arch, which it joins (12 mm. stage), the two then forming one continuous vessel. It is near this junction that the communication between the afferent and efferent vessels occurs (Text-fig. 1B). This communication, at first

small and single, later becomes plexiform with three or four openings into each vessel, and from this plexus, says Marshall, the definitive carotid labyrinth develops.

In *Rana*, the communication between the afferent and efferent vessels results from canalization of a plate of cells between them, not only in the first arch but also in the others. Maurer (1888) originally thought these cells come from the branchial epithelium, and besides entering into the formation of the labyrinth also form accessory epithelial bodies between the great vessels; according to Pischinger (1934), however, the cell-plates in *Rana* are not branchial in origin, but come from the endothelium of the two vessels—cells from the branchial epithelium, he says, take no part whatever in the formation of the carotid labyrinth. Mishima's results (1944) in the anurans *Rhacophorus arborea* and *Bufo formosus* substantially confirm these findings. In *B. vulgaris*, however, Pischinger found no cell-plates, and said that the communications arise here by a simple breaking-through of the apposed vessel walls.

Because there is no satisfactory account of the carotid labyrinth in English, and because of the common misconceptions and uncertainties regarding its structure and function, I have undertaken a detailed study of its gross anatomy and histological structure, and a brief study of its innervation, in a local species of anuran—*Hyla aurea*—as well as reviewing all the available relevant literature. Also I have briefly studied the organ in the native frog, *Leiopelma hochstetteri*. It has not been possible here to include a study of the development of the organ in either species.

#### MATERIAL AND METHODS

The investigations were mainly on *Hyla aurea*, of which I had a plentiful supply from the Physiology Department of this University, to whom my thanks are due.

The animals were killed by pithing, pinned in the supine position, and dissected, under a binocular microscope, to show the carotid labyrinth and related structures; the precise relations were confirmed by making a graphic reconstruction of the post-hyoid region and the floor of the mouth from a special series stained with iron-haematoxylin and picrofuchsin.

Labyrinths were fixed in Zenker's or Bouin's fluid, embedded in paraffin, serially sectioned at  $10\mu$  and stained with haematoxylin-eosin, azo-carmin and aniline blue, iron-haematoxylin and picrofuchsin, and with resorcin-fuchsin for elastic fibres. The post-hyoid region and buccal floor of one animal was fixed in Bodian's solution no. 4 (formol-formic), serially sectioned in paraffin at  $10\mu$ , and stained by Bodian's activated-protargol method.

Three wax-plate reconstructions ( $150\times$ ) of one labyrinth were made, taking every section in a  $10\mu$  H.-E. series. One (A) was of the walls of the organ; a second (B) was a cast of the interior of the common carotid artery, the main chamber, and the external carotid; while the third (C) was a cast of the tributaries of the internal carotid.

With permission of the Minister of Internal Affairs, I was able to study the labyrinth in the rare native frog, *Leiopelma hochstetteri*, which is rigorously protected. I dissected four of these animals, and one labyrinth was serially sectioned at  $10\mu$  and stained with iron-haematoxylin and picrofuchsin. I am greatly indebted to Mr S. G. Gittos of Warkworth, Auckland, for collecting these specimens for me.

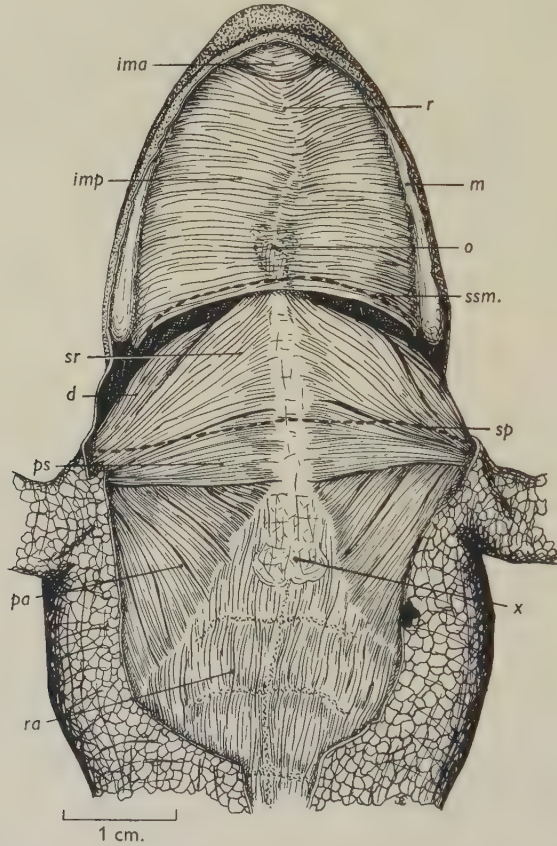


## RESULTS

I. *Hyla aurea*

The carotid labyrinth is exposed as follows:

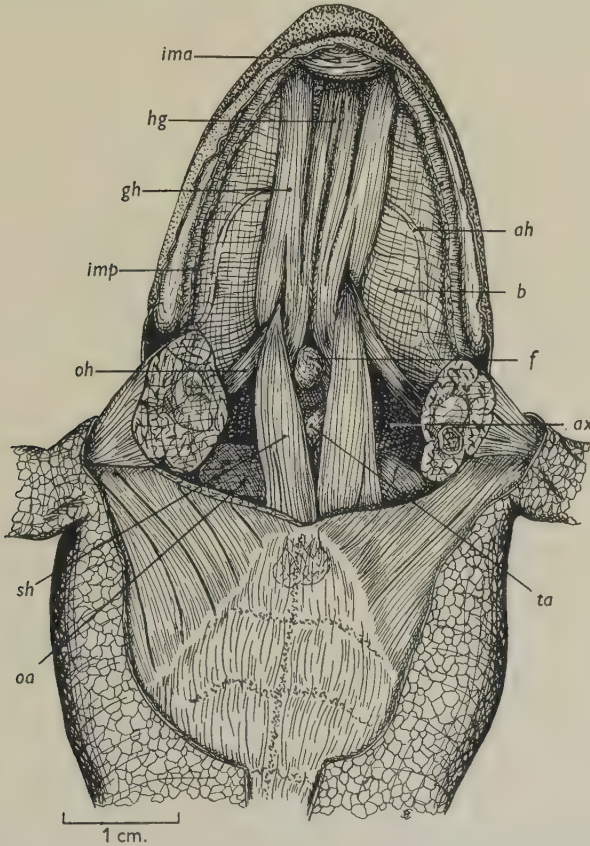
*Stage I.* The skin is incised along the mid-line, and across the pectoral region between the fore-limbs. The four flaps are then reflected, after cutting the two lymph-sac septa connecting the skin to the underlying muscles: anteriorly, the septum submaxillaris; more posteriorly, the septum pectoralis (Text-fig. 2). The anterior flaps are dissected back to the mandible and removed; the posterior flaps



Text-fig. 2. The superficial muscles exposed by stage I of the dissection.  
(Text-figures 2-4 were drawn from photographs.)

are reflected laterally and trimmed off to expose the entire pectoral region. This discloses the following muscles (Text-fig. 2): m. intermandibularis anterior, in the tip of the jaw; m. intermandibularis posterior, a muscular sheet between the mandibles; and on each side, m. deltoideus; m. sternoradialis; m. pectoralis, in two parts (pars sternalis and pars abdominalis); and finally the segmented m. rectus abdominis.

*Stage II.* The approach to the axillary region, where the labyrinth lies, now involves removal of the clavicle, coracoid and associated muscles. First of all, *m. intermandibularis posterior* is detached from the mandibles and removed, thus exposing the omosternum. Then a blade of the scissors is inserted between the two parts of *pectoralis*, about half a centimetre from the mid-line, and passed forwards beneath the *pars sternalis* and the underlying bones, being kept hard against the bones to prevent damage to underlying structures. The bones and muscles are

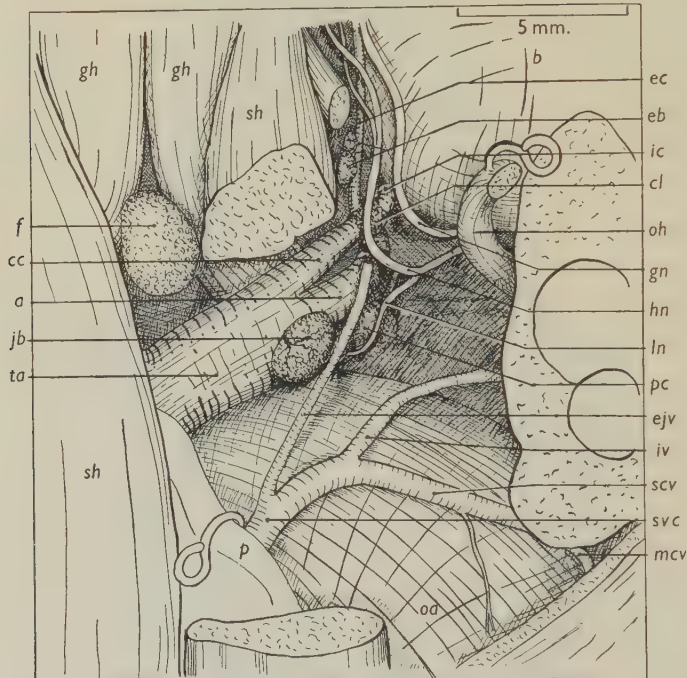


Text-fig. 3. The deeper muscles exposed by stage II of the dissection.

cleanly cut through, on both sides. Holding the omosternum, the whole centre-piece of the pectoral girdle is carefully dissected from the underlying sternohyoid muscles. The fore-limbs are pulled firmly apart and re-pinned, and so the axillary spaces are clearly exposed. The following muscles are now in view on each side (Text-fig. 3): hyoglossus muscle, alongside the mid-line; just lateral to and parallel with it, is geniohyoid arising caudally by two slips embracing the insertion of sternohyoid; sternohyoid, more caudally, also arises by two slips, from the dorsal surface of the xiphisternum and the rectus abdominis; and lastly, the slender omohyoid, which runs anteriorly, medially and ventrally to a common insertion

with sternohyoid into the hyoid bone. Between omohyoid and sternohyoid there is a triangular interval with its apex anteriorly: this is the focal point of the dissection, for in here lies the carotid labyrinth.

*Stage III.* Sternohyoid is now cut through caudally and, working from its medial border to protect the underlying structures, it is reflected rostrally and cut away cleanly between the slips of geniohyoid. The axillary space is thus fully exposed as in Text-fig. 4. Laterally, is the root of the fore-limb; medially, the pharynx and larynx; anteriorly, the posterior wall of the buccal cavity (extending out to the angle of the jaw); and posteriorly, m. obliquus abdominis separating the space from the pleuro-peritoneal cavity.



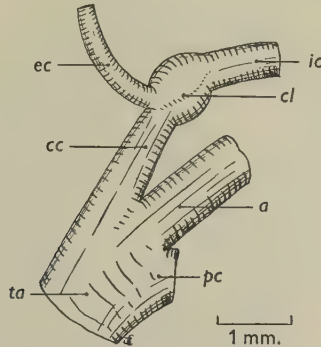
Text-fig. 4. The left axillary region exposed by stage III of the dissection. Anterior is above, and medial to the left. The left sternohyoid muscle (*sh*) has been cut away and the left omohyoid (*oh*) has been cut and retracted laterally. The anterior part of the external jugular vein (*ejv*) has been cut away. This animal was anomalous in that the hypoglossal nerve (*hn*) passed forwards deep to the omohyoid; otherwise the course is quite typical.

In the mid-line, the bulbus arteriosus emerges from the pericardium and divides at once into two branches, the right and left truncus arteriosus. Each runs rostrally and laterally and soon divides into three main arteries. The most posterior is the pulmo-cutaneous artery, arching first dorsally and then caudally. Anterior to this is the aorta, which arches dorsally and somewhat rostrally (it finally turns medially and caudally). Just lateral to the point where these two arteries first diverge there is a prominent gland-like structure—the jugular body—which, as an outstanding feature of the axilla, is a very useful landmark, although, in certain species, it



should be noted, it is more anterior and may even be rostral to and medial to the carotid labyrinth (Romeis, 1926). Finally, the most anterior branch of the truncus is the common carotid artery, which is slightly narrower than the others and runs anteriorly, as well as dorsally and laterally, thus gradually diverging from the aorta; it ends, after a course of 1–2 mm., in a more or less well-marked swelling—the carotid labyrinth (Text-fig. 5).

The *carotid labyrinth* is about 1 mm. long and is characterized by its dark pigmentation. From it spring two arteries: the internal carotid, which, as the apparent continuation of the common carotid, continues to arch dorsally; and the smaller

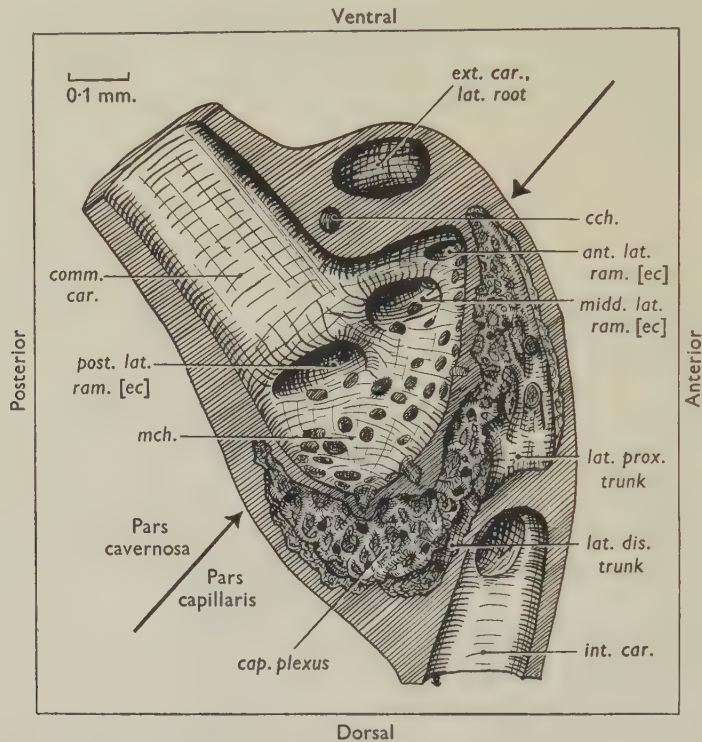


Text-fig. 5. The great vessels of the left side, seen from the ventral aspect.  
(Drawn from a camera lucida sketch.)

external carotid, which leaves the rostral aspect of the labyrinth close to the termination of the common carotid, and, after passing a little medially, runs anteriorly under omohyoid into the floor of the mouth (Text-fig. 4). The hypoglossal nerve, emerging laterally from under omohyoid, sweeps medially and ventrally over the aorta, then rostrally over the carotid labyrinth, finally dividing into two branches; these continue rostrally, ventral to the insertion of omohyoid, and then over the external carotid to lie medial to it. The glossopharyngeal nerve at first follows a similar course to the hypoglossal, although a little more anteriorly and laterally. It crosses the origin of the internal carotid to enter the floor of the mouth, lateral to the external carotid and beneath omohyoid. The laryngeal branch of the vagus nerve lies more deeply and, passing caudally and medially, winds round the lateral aspect of the pulmo-cutaneous trunk.

The veins of the axilla (Text-fig. 4) are delicate and thin-walled. They comprise: the subclavian vein, commencing laterally at the union of the brachial and musculo-cutaneous veins and sweeping medially over the caudal wall of the space; the innominate vein, coming from the junction of the subscapular with the internal jugular to join the subclavian vein; and the external jugular, which begins in the floor of the mouth and runs directly caudally, ventral to the carotid labyrinth and the IXth and XIIth nerves, and lateral to the jugular body—it finally joins the subclavian vein to form the superior vena cava, which opens into the sinus venosus under cover of the projecting pericardium.

The epithelial bodies, or parathyroid glands (Text-fig. 4), two on each side, lie just medial to the external carotid artery a little in front of the carotid labyrinth. A prominent fat-body is often present between the origins of the hyoglossus muscles and is possibly a seasonal phenomenon (Text-figs. 3, 4).



Text-fig. 6. The lateral half of a right carotid labyrinth looking from the medial aspect. The lateral portion of the capillary plexus (*cap. plexus*) and the lateral tributaries of the internal carotid are shown. The organ is divided into its two parts, *pars cavernosa* proximally and *pars capillaris* distally, at the arbitrary plane indicated by the arrows. (Text-figures 6–11 are from tracings, made on a dioptograph, of the wax-plate reconstructions B and C.)

### Morphology

#### The carotid labyrinth

Superficially, the carotid labyrinth in *Hyla* is a distinct swelling 1 mm. long and 0.6 mm. across, right at the bifurcation of the common carotid artery, which reaches it at its proximal pole.\* The smaller external carotid leaves the labyrinth just anterior to the common carotid and passes medially and anteriorly, the two vessels

\* The terms of reference used in describing the carotid labyrinth are not always consistent and are sometimes confusing, so that difficulties are encountered when comparing the works of different authors. Zimmermann, for instance, has described the organ as if seen from the ventral aspect whereas I have regarded it as a portion of the arterial arch III, which passes from ventral to dorsal. Thus the proximal pole, which I describe as ventral, he called medial, and the distal pole, which I consider as dorsal, he described as lateral. Consequently, the surfaces which he calls ventral and dorsal, I have called lateral and medial.

forming an angle with each other of  $90^\circ$  or even less (Text-fig. 5). Leaving the opposite (distal) pole of the labyrinth is the internal carotid artery.

Although superficially the external and internal carotids appear to arise from the opposite poles of the labyrinth in a perfectly simple and straightforward manner, their actual origins internally are, in fact, very complex; for the common carotid artery does not lead directly into either vessel: shortly after entering the swelling it dilates, somewhat asymmetrically, into a large chamber (the main chamber) which discharges on the one hand into the external carotid by six separate channels, and on the other into the internal carotid through a mass of capillaries and a system of collecting vessels (Text-fig. 6).

The common carotid artery, when it reaches the labyrinth, has a bore of about 0.25 mm.; it runs into the organ for about 0.2 mm. before actually opening into the main chamber; this opening is slightly oblique so that the posterior wall of the common carotid is longer than the anterior (Text-figs. 6, 7).

The main chamber is 0.5 mm. long and about 0.4 mm. across. *Proximally*, is the opening into it of the common carotid posteriorly, while more anteriorly are the openings out of it of the external carotid. *Distally*, are a host of small orifices leading to a capillary plexus. In fact, the entire labyrinth may be said to present two parts—proximal and distal. The proximal part ('pars cavernosa' of Zimmermann) comprises the termination of the common carotid, the origin of the external carotid, and the associated part of the main chamber. The distal part ('pars capillaris' of Zimmermann) contains the capillary plexus and the associated portion of the main chamber, as well as the various tributaries of the internal carotid which drain the capillary plexus (Text-fig. 6; Pl. 1, fig. 1).

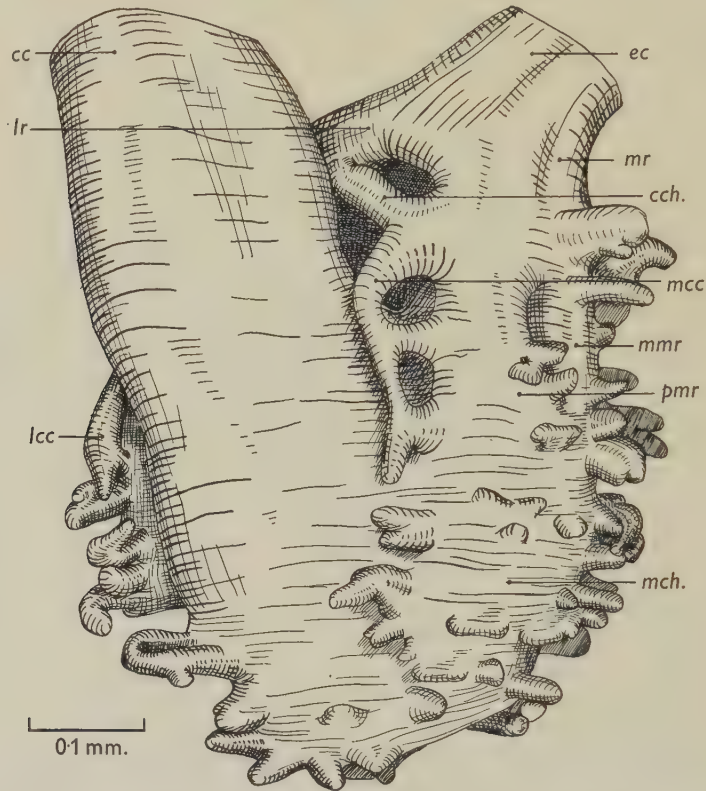
The external carotid artery arises from the main chamber by three orifices on each side—posterior, middle and anterior. The anterior openings are the most ventral while the posterior ones, which flank the opening of the common carotid into the chamber, lie most dorsally (Text-fig. 6). Each opening leads *ventrally*, that is *recurrently*, into a corresponding *ramus*. Of these, the posterior on each side is the longest; it arches ventrally to join the middle ramus, which arises from the chamber just anterior and ventral to it. The common trunk so formed is then joined by the smaller anterior ramus to form the lateral or medial *root* of the external carotid as the case may be (Text-fig. 8). The lateral root now arches over the proximal end of the main chamber, anterior to the common carotid, and unites with the shorter and more vertical medial root to form the external carotid artery (Text-fig. 8). This then leaves the organ running medially and ventrally. In addition, the two roots may communicate by a small accessory channel in front of the common carotid (Text-fig. 7).

Near their origins, the rami give off a number of small vessels which pass to the capillary plexus (Text-figs. 6, 8). Furthermore, it is likely that they may present minor variations; here, for instance, the lateral anterior ramus arose by union of two smaller trunks, while each posterior ramus had a small collateral channel of varying length each of which also sent a branch dorsally to the capillary plexus (Text-figs. 7, 8).

The diameters of these vessels are approximately as follows: the rami, 0.08–0.1 mm.; the roots, 0.12 mm.; the external carotid itself, 0.14 mm. The accessory communicating channel and the collateral channels were only 30–40  $\mu$  wide.



The capillary plexus is a true rete mirabile, between the main chamber on the one hand and the internal carotid artery on the other. It forms, as it were, a cap fitting over the distal half of the main chamber. It is thickest (0.2 mm.) at its centre and tapers peripherally. Its concave, afferent aspect faces towards the main chamber, and its convex, efferent aspect is related to the tributaries of the internal carotid (Text-fig. 6).



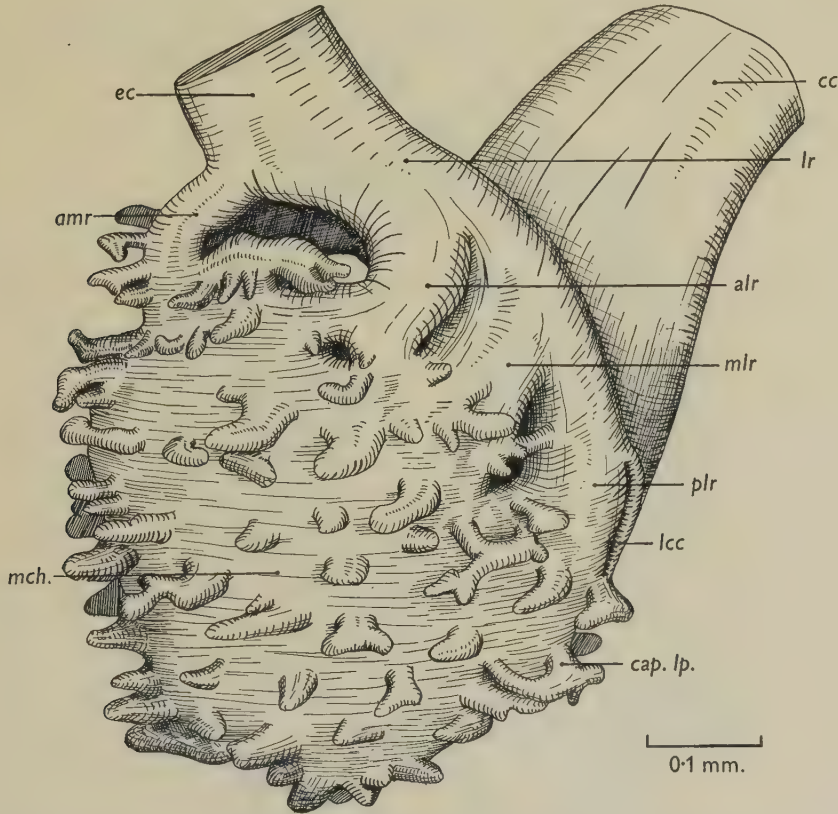
Text-fig. 7. The common carotid artery (cc), main chamber (mch.) and external carotid artery (ec), Postero-medial aspect seen from a little dorsally. In Text-fig. 7-11, dorsal is below.

A host of afferent vessels of small bore ( $20-40\mu$ ) leave the distal part of the main chamber and radiate out into the plexus. They soon begin to divide and anastomose freely with each other, often forming loops, from which secondary branches proceed (Text-fig. 8). As the result of continued division and anastomosis a dense capillary meshwork is formed, the smallest channels of which are about  $10\mu$  wide and only  $20-30\mu$  long. Towards the convexity of the plexus the capillaries progressively reunite into larger efferent vessels ( $20-30\mu$ ) which finally drain into the various tributaries of the internal carotid.

The *internal carotid artery* is fed by a system of tributaries which converge on it over the convex surface of the capillary mass, from its rim; they receive the efferent vessels of the plexus and eventually form four main trunks, two proximal and two

distal, which join, just anterior to the dorsal pole of the capillary plexus, to form the internal carotid (Text-fig. 6).

Efferent vessels arising near the rim of the two anterior quadrants of the capillary mass unite to form four or five larger vessels on each side; these curve dorsally in the anterior wall of the organ, draining as they go the subjacent parts of the plexus.



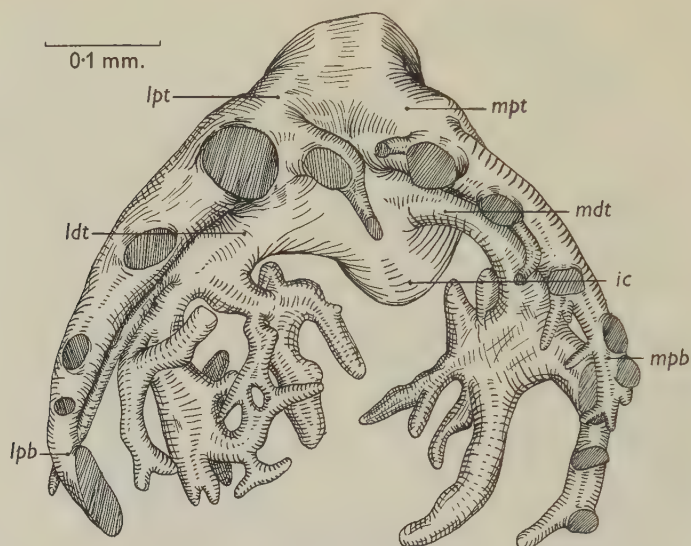
Text-fig. 8. The common carotid artery (*cc*), main chamber (*mch.*) and external carotid artery (*ec*). Antero-lateral aspect seen from a little ventrally.

Opposite the distal pole of the main chamber, the most lateral and the most medial of these tributaries turn towards each other and, after receiving in succession the three or four others on each side, they form respectively lateral and medial *proximal trunks*, which, after a short course (0.1 mm.), finally join each other (Text-figs. 9, 10).

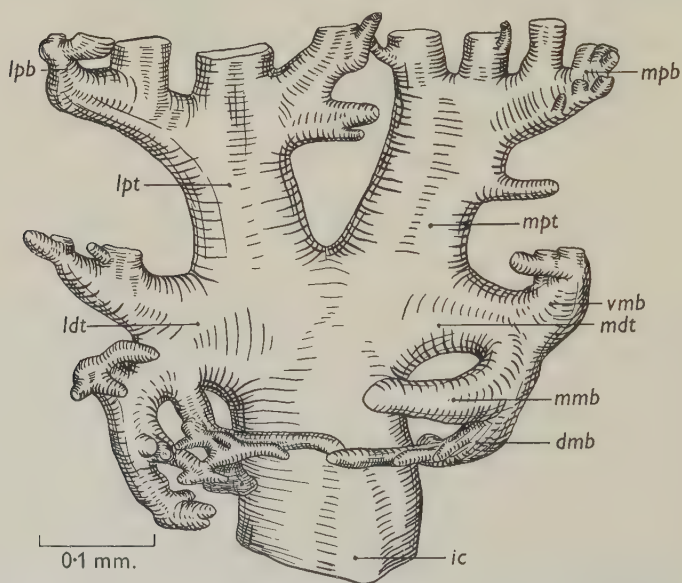
The vessels from the dorsal part of the plexus form, on each side, three main tributaries—ventral, middle and dorsal. These, in turn, join to form lateral and medial *distal trunks*, which run transversely for about 0.1 mm. to join the united proximal trunks and thus form the internal carotid proper (Text-fig. 11), which then leaves the organ to pass almost directly dorsally. The main trunks are from 0.07 to 0.11 mm. in diameter, while that of the internal carotid is 0.2 mm.

*Histology*

The common carotid is a musculo-elastic artery. It has a distinct internal elastic lamina; its media has numerous elastic fibres and much smooth muscle arranged in a thick, inner circular layer and a thinner, outer longitudinal one; finally, there is a thin fibrous adventitia. The external carotid artery has a thinner muscular media



Text-fig. 9. The tributaries of the internal carotid artery seen from ventrally.

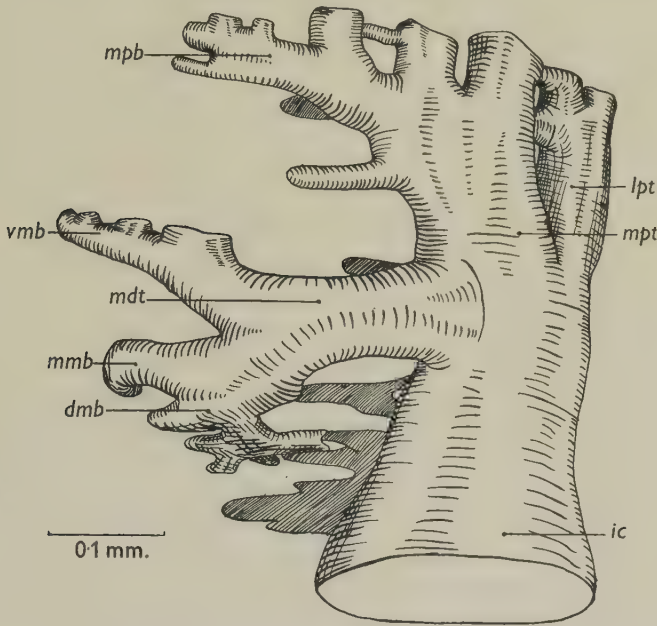


Text-fig. 10. The tributaries of the internal carotid artery, seen from posteriorly.



with no internal elastic lamina. The muscular tissue of these two vessels gradually disappears in the proximal part of the main chamber, but the fibro-elastic tissue persists. The wall of the main chamber related to the capillary mass is strongly fibro-elastic, the fibres running mainly transversely, skirting round the openings of the afferent vessels of the rete (Pl. 1, fig. 2). Towards the distal pole of the chamber, the fibrous tissue of its wall is more delicate.

The interstitial stroma of the capillary plexus consists of a delicate collagenous tissue interwoven with fine elastic fibres; there are very few smooth muscle fibres. The nuclei of the fibrocytes are ovoid and stain only moderately darkly. The nuclear



Text-fig. 11. The tributaries of the internal carotid artery. Medial aspect seen from a little anteriorly and dorsally.

picture here is complicated by the plexiform nature of the region and by the fact that the fibrocyte nuclei are often overlaid by large, pale, ovoid endothelial nuclei, and also, sometimes, by blood cells (Pl. 1, fig. 3). At its convex surface, the rete is more definitely fibrous and the elastic fibres are generally thicker here.

The tributaries of the internal carotid have thin fibro-elastic walls, but the main vessel soon assumes a structure similar to that of the common carotid, with an internal elastic lamina and a substantial muscular media.

The whole organ has a thin fibrous adventitia and lies in a loose, adipose connective tissue. In this tissue are scattered clumps of melanophores, while similar clumps are also associated with the tributaries of the internal carotid.

Nowhere in the organ have I seen cells of an epithelioid nature; and I believe that these do not exist in *Hyla aurea* and that only vascular tissues are present here.

Thus, in contrast to the main arteries, the carotid labyrinth is predominantly

fibro-elastic; the muscular coats of the common and external carotids disappear in the adjacent parts of the organ while muscular tissue does not reappear in the internal carotid until it is fully established. The walls of the main chamber and of the tributaries of the internal carotid are clearly fibrous while the interstitial tissue of the capillary plexus is much more delicate in nature.

### *Innervation*

The innervation of the carotid labyrinth is very sparse. Axons run in it to leave its lateral aspect as three or four bundles, each of four to six axons, which, after a very short course antero-laterally, reach a microganglion; from this a fine nerve runs about 1 mm. rostrally and laterally to the glossopharyngeal nerve, which it joins by turning back on itself. Whether the nerve bundles leaving the labyrinth subserve a motor or a sensory function, or both, I cannot say, for I have made no special attempt to demonstrate nerve-endings in the organ, nor have I been able to carry out experimental procedures to determine the situation of the cell-bodies of the axons.

### II. *Leiopelma hochstetteri*

*Leiopelma* is smaller than *Hyla*, with a body length of only about 4 cm., as against 8–10 cm. in *Hyla*. The approach to the axilla and its gross anatomy are much the same as in *Hyla*. However, within the axilla the great vessels are relatively longer and the common carotid arteries pass almost directly laterally; the carotid labyrinth, too, although comparatively long (0.8 mm.) is slimmer than in *Hyla*, and on this account appears more elongated; it is also much more darkly pigmented—indeed, the walls of the great arteries generally, are speckled by clumps of pigment cells which are also scattered throughout the connective tissue of the axilla.

Although in one specimen of *Leiopelma* the arrangement of the vessels of the labyrinth closely resembled that in *Hyla*, in three others, the most striking feature of the external appearance of the organ was the fact that, in contrast to *Hyla*, where the external carotid parts company with the common carotid almost at once, here the two arteries ran side by side, with what was virtually a common wall, for a considerable distance (up to 1 mm.), before the external carotid turned rostrally away from the common carotid. In spite of the fact that the two vessels are thus parallel, the blood in them is, of course, running in opposite directions.

Although I have not been able to complete a detailed study of the internal anatomy and histological structure of the organ, the carotid labyrinth in *Leiopelma* is, as far as I can tell, quite comparable with that of *Hyla*.

### DISCUSSION

The carotid labyrinth of *Hyla aurea* is quite similar to that in those anurans in which it has been studied in detail (Zimmermann, 1887; Pischinger, 1934; Eloff, 1935; de Boissezon, 1939; Ishida, 1954), for in all of them the common carotid extends for some distance into the organ where it gives rise proximally to several channels on each side; these unite over the common carotid to form the external carotid, which has a somewhat recurrent course—thus the roots of the external

carotid straddle the common carotid 'like a rider astride a horse' (Brücke, 1852). In *Hyla*, however, the roots are not connected caudally to form a vascular ring right around the common carotid, as they are in *Rana* (Zimmermann) and *Bufo* (Pischinger). Distally, the common carotid ends abruptly, well within the labyrinth, by resolving itself into a host of small vessels which form a dense capillary plexus from which in turn a system of collecting vessels drains the blood into the internal carotid artery. Histologically, also, the labyrinth in *Hyla* closely resembles that of other anurans. As will be discussed more fully below, the capillary plexus is notable for its paucity of muscular tissue and for the delicate nature of its connective tissue, while the innervation of the organ is exceedingly sparse.

In view of the great structural complexity of the labyrinth, particularly in contrast to the simple nature of the comparable region in the mammals (carotid sinus), it is little wonder that the question of its functional significance has excited considerable interest, controversy and speculation. As we have seen, it has been variously regarded as a gland (which it obviously is not), an accessory heart, a form of resistance to the blood flow, a chemo-receptor, a pressor-receptor, as well as a mechanism for controlling the volume of blood entering the internal carotid. Yet, as I propose to show, none of these suggestions is free from objection:

(1) Even though Noble, as recently as 1931, has suggested that the organ 'steadies the (blood) pressure by continuing to contract between (heart) beats' (p. 191), it seems quite certain that it is in no sense an accessory heart; not only is its gross structure quite inconsistent with such a function but it obviously is not sufficiently muscular. Although, with some stains, the histological appearance of the capillary plexus may be misleading in this respect (Palme, for instance, thought it was completely muscular), there is no doubt that it is predominantly fibro-elastic, and that the muscle-fibres present are so few that they would be quite ineffectual against the pressure in the vessels (30–35 mm. Hg).

(2) It was Brücke's belief (1852) that the venous and arterial bloods leaving the heart are differentially distributed, the most venous to the pulmo-cutaneous trunks and the most richly oxygenated to the common carotids, so that the brain receives only blood of the highest *quality*. According to this hypothesis, the capillary plexus presents to the earlier (venous) outflow a resistance which is only overcome later in ventricular systole when oxygenated blood is being ejected. It is of course essential to this hypothesis (which had the authoritative support of Zimmermann and which is still widely held) that the venous and arterial bloods stay unmixed in the single ventricle, and pass out through the bulbous in sequence. Even on *a priori* grounds, this hypothesis would seem very unlikely, for not only does a lot of reoxygenated blood from the musculo-cutaneous veins mix with the general venous blood, but the oxygen tensions of the different bloods would immediately tend to equalize in the ventricle so that there would not be much advantage in their remaining segregated. However, there is no need to fall back on this line of reasoning, for Vandervael (1933) has shown experimentally that there is, in fact, considerable mixing of blood in the ventricle, and that therefore the segregation hypothesis is no longer tenable. This has since been confirmed arteriographically by Foxon & Walls (1947) who were also able to show that radio-opaque material, injected into a musculo-cutaneous vein, and which should, according to the hypothesis, first outline the pulmo-



cutaneous arteries, appears, in fact, first in the aortae—by the time the pulmonary vessels are filled so also are the carotids. Thus, it is clear that the function of the labyrinth cannot be to aid the carotids in getting a preferential supply of richly oxygenated blood.

(3) Maurer (1888, 1899) believed the carotid labyrinth of the Amphibia to be homologous with the carotid body of mammals, because he thought both contain cells of branchial origin. Because of this, and because the mammalian carotid body has since come to be regarded as a chemo-receptor, some have attributed a similar role to the carotid labyrinth. However, we now know that, in mammals, although the actual source of the specific (epithelioid) cells of the carotid body is still debated, they are definitely not of branchial origin. In the *Anura*, too, as Pischinger (1934) and Mishima (1944) have shown, the cell mass, which in the tadpole develops at the site of the future labyrinth and which Maurer regarded as of branchial origin, is undoubtedly derived from the endothelium of the vessels of the first branchial arch. Furthermore, such cell-masses are not peculiar to the 3rd arch alone, for in *Rana*, for instance, identical cells appear in the other arches too. Moreover, in some species (e.g. *Bufo vulgaris*) the labyrinth develops without any such cell-mass appearing at all (Pischinger).

Thus it seems definite that the cells which enter *initially* into the formation of the labyrinth are all of vascular origin. However, it is not impossible that this early primordium may be invaded later by extrinsic cells of a chemo-receptor nature. Thus, de Boissezon (1939) has described, in the pars capillaris, cells with large, clear nuclei and a clear, vacuolated cytoplasm, which he regarded as 'comparable... to the clear cells of the carotid body which we have described in certain mammals and... to those which Granel (1927) described in the pseudobranchia of the fishes' (p. 151). And again, Chowdhary (1951) (following Hollinshead's staining methods, 1943, 1945, for the mammalian carotid body) has identified cells in the carotid labyrinth of *Rana tigrina* which, he says, have a marked similarity to mammalian carotid body cells, but which 'occur sparingly and only in the pars arterialis'. Pischinger (1934), however, was unable to find any such cells, nor have I myself ever seen them. Indeed, the carotid labyrinth is so different from the carotid body of higher animals, both in its microscopic structure and its architecture, that it is very unlikely that it can be solely a chemo-receptor, and I believe such a function in no way explains its complex internal structure.

(4) Is the organ, then, a pressor-receptor? As noted, Meyer (1927) demonstrated depressor fibres in the glossopharyngeal nerve, entering it near the great vessels; but whether or not these fibres proceed from the labyrinth he left undecided. Others, however, have jumped to the conclusion that they do and that therefore the carotid labyrinth is functionally comparable with the mammalian carotid sinus (Ask-Upmark, 1935, p. 103). This also appears to be the view of Neil *et al.* (1950) who describe, in *R. temporaria*, a fine 'carotid sinus nerve' arising from the labyrinth and going to the glossopharyngeal and which, from its course and relations, is clearly the same one as I observed in *Hyla*. Although they obtained afferent spike potentials from this nerve between heart beats, this was achieved only by so raising the blood pressure (by filling the sinus venosus at a pressure—'by no means normal in the frog'—of 2.5 cm. H<sub>2</sub>O) as to cause 'a marked distension of the arterial system'

(pp. 342-3). At times they had even to tie off the opposite truncus to ensure an adequate blood flow to the side being studied. Clearly these experimental conditions are quite abnormal, and although Neil *et al.* claim that their results may be correlated with Meyer's, they concede that they had 'no evidence of the existence of a reflexogenic mechanism' and add 'we have only found the stretch receptors firing under conditions of raised arterial pressure and cannot hazard an opinion as to whether this mechanism is tonically active under normal circulatory conditions' (p. 348). Since no histological controls were carried out, the nature of the end-organs concerned was not determined, nor was it shown that the impulses they recorded could produce any fall in blood pressure—they may well have been pain impulses.

Just as there is, as yet, no satisfactory *physiological* evidence to suggest that the carotid labyrinth has a pressor-receptor function, there is similarly no *histological* evidence for such a function—as de Boissezon (1939), discussing the possibility of a reflex nervous mechanism originating in the organ puts it: 'il y a là une question qui, au point de vue histologique, reste encore obscure pour nous' (p. 152). Eloff (1935), too, considered the organ as too strongly elastic to permit the passive distension of which the carotid sinus is capable. Furthermore, Palme (1934) points out that, while the innervation of the capillary plexus is poor, that of the termination of the common carotid and the great vessels generally, is much richer, which 'hauptsächlich wohl als sensible Fasern ansprechen darf' (p. 416); this would seem to indicate that, rather than the labyrinth, the great vessels themselves are a more likely site for the pressor-receptors. At least, it is certain that a structure like the labyrinth is not essential for pressor-reception, for the depressor fibres in the vagus nerve of the frog (Kuno & von Brücke, 1914) obviously have no comparable specialized site of origin. Indeed, to ascribe to the carotid labyrinth a pressor-receptor function is to attribute to a very complex organ a function which, in all other cases, is subserved by a very much simpler apparatus (such as the mammalian carotid sinus), and so to fail to account adequately for its great complexity.

(5) To account for his suggestion that the carotid labyrinth may regulate the *volume* of blood entering the internal carotid, Pischinger (1934) pointed to the following considerations (p. 52): first, that the organ is present only in species which are truly amphibious; secondly, that it develops in these when they become terrestrial; thirdly, that it is absent in the *Gymnophiona* 'which according to Brehm never take to the water'. Because truly amphibious amphibians are subject to sudden pressure changes, he believes that a regulating device or throttle ('Drosselorgan'), such as the labyrinth, is necessary to prevent overloading of the territory of the internal carotid. Any excess of blood dammed back in the labyrinth by this 'throttle mechanism' can be more readily diverted into the external carotid because of the recurrent course of its roots of origin; indeed, Pischinger suggested that 'dieser Verlauf der Kanäle (i.e. roots of origin of the external carotid) kann nur durch eine Rückstauung des Blutes durch das Labyrinth der Interna bedingt sein, die schon während der Organogenese vorhanden ist' (p. 52).

Unfortunately, there are certain defects in this hypothesis. Amphibious animals do experience sudden pressure changes, as Pischinger stated, but these must always act equally on all body fluids—the cranial contents would not be especially affected by changes in the external pressure, because simultaneous increases in the venous

and cerebro-spinal fluid pressures will maintain the state of equilibrium within the cranium; therefore, a special device on the carotid arch to achieve this equilibrium is quite unnecessary. Pischinger also said that the organ is well-adapted for regulating the amount of blood entering the internal carotid, because 'infolge der vorhandenen muskulären und elastischen Elemente, der Gesamtquerschnitt aller Lichtungen weitgehend variabel ist' (p. 51); but according to nearly all accounts I have seen, including Pischinger's own (p. 47), the capillary region is practically devoid of muscle fibres, and furthermore, such an active control would surely require a considerable innervation, a necessity which Pischinger appears to have overlooked. A final objection is that one genus of Gymnophiona (*Typhlonectes*) is said to be aquatic (Noble, 1931); while the African toad, *Breviceps*, although never taking to the water at any stage of its life history, has, nevertheless, a carotid labyrinth (Eloff, 1935).

De Boissezon (1939), also, believed the labyrinth to be concerned in the control of cerebral blood pressure. He stated that the findings of Granel (1927) and Ask-Upmark (1935) 'show the constancy of the apparatus regulating the cerebral blood pressure in all the vertebrate series, and the role of the carotid plexus in the Amphibia is beyond doubt' (p. 147). According to him, the mechanism is twofold: first, the capillary plexus slows up the blood flow; and secondly, the elastic nature of the organ allows it to distend under an increase of pressure and then to recoil. Although the organ must undoubtedly modify the blood pressure in the internal carotid, such purely passive, mechanical actions cannot possibly 'control' it in the usual physiological sense; they can only cause it to vary directly with the pressure in the common carotid—as the latter rises so also must the pressure in the internal carotid.

We see, therefore, that, of the various hypotheses put forward to explain the significance of this highly specialized structure in the Amphibia, some are obviously quite untenable, and not one is entirely free from objection. It cannot be an accessory heart, nor does it facilitate differential distribution of the blood, nor can it actively regulate the volume of blood going to the internal carotid, while its structure seems unnecessarily complex for a pure chemo- or pressor-receptor. What then can be its function? Is there any aspect which has been overlooked or not fully appraised, in the past, which ought to be taken into account in considering this question?

One striking fact about the amphibian carotid labyrinth, as Pischinger clearly perceived, is the peculiar disposition of the external carotid: this vessel forms an acute angle not, as one might expect, with the *internal* carotid, but with the *common* carotid; that is to say, the common carotid bifurcates at an obtuse angle, so that the external carotid leaves it in just the reverse direction to that which we would expect. This has been described or illustrated in many detailed accounts of Anura and Urodela (Ecker & Wiedersheim, 1881; Zimmermann, 1887; Scholz, 1933; Francis, 1934; Pischinger, 1934); it is particularly well marked in *Leiopelma* and *Salamandra*, where the external carotid runs back close alongside the parent common carotid for an appreciable distance before turning rostrally. Further, it appears that in the Gymnophiona, where the bifurcation is not obtuse but is a normal acute-angled one, there is no carotid labyrinth at all (Boas, 1883). These facts obviously suggest that there is a significant relationship between the angle of bifurcation of the common carotid and the presence or absence of a carotid labyrinth.



In the tadpole, the external carotid is at first quite independent of the other vessels of the first branchial arch; soon it grows caudally, laterally and dorsally, to become directly continuous with the efferent vessel of this arch (Marshall, 1893; Pischinger, 1934, figs. 2, 3). The gill circulation at this stage being functional, the blood entering the efferent vessel can flow equally readily in either direction—dorsally into the dorsal aorta, or ventrally into the external carotid. After metamorphosis, however, the afferent vessel develops a new and substantial communication with the ventral part of the efferent vessel; this communication is so disposed that the main blood flow will now pass from the truncus directly to the dorsal aorta through what is virtually one continuous channel, while the external carotid leaves the arch somewhat ventrally in a direction recurrent to that in which the main blood stream is flowing (Text-fig. 1).

In view of this, the question arises as to whether such an arrangement might put the external carotid at a considerable disadvantage. That this might be so seems likely on general hydrodynamic principles, for Evans (1949) says that 'when a tube gives off a branch, the pressure exerted in the branch by a fluid *flowing along* the tube will depend upon the angle at which the branch leaves the main vessel. If the branch leaves at right angles, then the hydrostatic pressure in the branch is equal to the lateral pressure on the walls of the main vessel. If the branch inclines at an acute angle to the direction of flow, then owing to the kinetic energy of the moving column of fluid, the pressure in (the branch) will be greater than the lateral pressure in the main stem, while if at an obtuse angle it will be less' (p. 617). Thus if the external carotid arises, as it does in the frog, at an obtuse angle to the direction of the main blood flow, and if it is in simple communication with the main arch, the filling of the vessel would be considerably hampered as compared with branches which arise from the great vessels at acute- or right-angles. An obvious way to overcome such a disadvantage and to increase the flow into the external carotid would be to interpose on the arch a device whereby the blood is turned back into the external carotid without prejudicing the flow into the internal carotid. That the carotid labyrinth is admirably adapted to serve both these purposes is clear from the following: the various openings leading into the external carotid are so placed that not only are they in a most favourable position to receive blood which is reflected from the more distal parts of the main chamber back along its side walls, but, at the same time, they are sheltered from the inrushing blood from the common carotid, the kinetic energy of which would otherwise cause a decrease of pressure in the external carotid. In other words, I suggest that within the main chamber of the labyrinth in the living animal there is an axial stream (or 'core') of inflowing blood around which, anteriorly, medially and laterally, is a peripheral layer of reflux blood feeding back into the external carotid on the one hand and, on the other, discharging, together with the 'core' stream, into the capillary plexus and hence the internal carotid.

The conception of the labyrinth as an important adjunct to the maintenance of an adequate blood flow in the external carotid may be supported by several arguments. First, it does not require, as does Pischinger's theory, that all truly amphibious Amphibia possess a carotid labyrinth. Secondly, the labyrinth is not a primary development in the tadpole, as one might well expect if it were a chemoreceptor, but arises secondarily, it seems, solely as a consequence of the

vascular changes occurring at metamorphosis. Thirdly, no artery corresponding to the external carotid develops in any of the other functional arterial arches, and in these no labyrinth forms, although the other vascular arrangements here are exactly the same as in the first arch, where a labyrinth does develop.

Moreover, the gross and microscopical structure of the organ is entirely consistent with the function suggested, which is, of course, purely a passive one. The capillary plexus is fibro-elastic, although not markedly so, for the very fine fibres are often difficult to make out and none of the nuclei are greatly compressed. Nor is it necessary for this interstitial tissue to be strong, for it is supported equally on all sides by the pressure in the constituent vessels, and thus has merely to withstand the kinetic force of the flowing blood, much of which, in any case, will have been absorbed by the more strongly fibrous walls of the main chamber. Furthermore, the capillary channels are very short, which is understandable if, as is quite clearly the case, they are not serving as true capillaries, but are merely performing a mechanical function. A passive function of this sort would obviously not necessitate a large nerve supply, which in fact the organ does not have, although the significance of the few fibres which are present is still in doubt. Furthermore, it is a significant fact that others have likewise ascribed a purely passive function to the organ (Brücke, 1852; Zimmermann, 1887; de Boissezon, 1939); their error, I believe, has been not in the actual function which they have claimed for it, but in the reason for which they have considered it to exist.

Finally, there is one further question which we must consider here. Where precisely on the arterial arch does the carotid labyrinth develop? As we have seen, Marshall (1893) believed that it develops from a plexiform elaboration of the primitive communication between the afferent and efferent vessels of the arch. However, Pischinger (1934) has stated that, in *Rana*, similar plexiform communications develop in the other arches as well, where, of course, they do not persist; and it seems certain too, that no such plexiform connexions develop in *Bufo vulgaris*, where the communication results simply from a breaking down of adjacent vessel walls. Furthermore, if the external carotid retains its primitive connexion with the efferent vessel (and there is no reason to suppose that it does not), it is clear, from the structure of the definitive organ, that it must be largely developed in the region where the efferent vessel joins with the external carotid, and that the capillary plexus must be formed almost entirely within the lumen of the efferent vessel (Text-fig. 1C). Mishima (1944) states that in *B. formosus* the organ develops by numerous processes growing into the lumen from the walls of the common, external and internal carotids, and it seems probable that those from the common and external carotids are principally involved in the production of the complicated origin of the external carotid, while those from the internal carotid give rise to the capillary plexus. But of course, this point must remain in doubt until the development of the organ is re-examined in the light of the conception put forward here. Furthermore, there is a pressing need for at least brief studies of as many species of Amphibia as possible, for the conception depends on one critical assumption: that in no amphibian in which the organ is absent does the common carotid bifurcate obtusely, and conversely, wherever the labyrinth is found, the bifurcation will always be obtuse.

## SUMMARY

1. A description has been given of the gross and microscopical anatomy of the carotid labyrinth in *Hyla aurea*. This organ, which comprises a swelling right at the bifurcation of the common carotid artery, is structurally very complex but histologically quite unspecialized. The common carotid opens into a main cavity within the swelling, and from this some six openings lead away recurrently into the external carotid, which forms an *acute* angle with the *common* carotid, and *not* with the internal carotid. This condition is found in the extreme in *Leiopelma hochstetteri*. Distally the main chamber gives off a host of channels leading to a compact rete mirabile which quickly resolves itself, through a system of collecting vessels, into the internal carotid. The muscular coats of the three carotid arteries gradually disappear in the related parts of the organ. The walls of the main chamber and the collecting vessels are fibro-elastic, and the interstitial stroma of the rete is a delicate collagenous and elastic connective tissue. The organ is very sparsely innervated.

2. Several hypotheses have previously been put forward to explain the significance of the carotid labyrinth in the Amphibia. However, after reviewing the relevant literature, and in the light of my own findings, I am convinced that the organ is certainly not an accessory heart, nor does it aid the differential distribution of the blood (an old hypothesis now untenable), nor, again, can it regulate the volume of blood entering the internal carotid. Furthermore, its structure is unnecessarily complex for it to be purely a chemo- or pressor-receptor.

3. I have suggested that the carotid labyrinth develops at metamorphosis to ensure that the external carotid artery receives an adequate supply of blood at a reasonable pressure, because this vessel, on account of its development, is found forming an *acute* angle with its parent vessel—the common carotid—and hence it leaves this recurrently; it is, therefore, hydrodynamically at a disadvantage. There are a considerable number of arguments which support this conception.

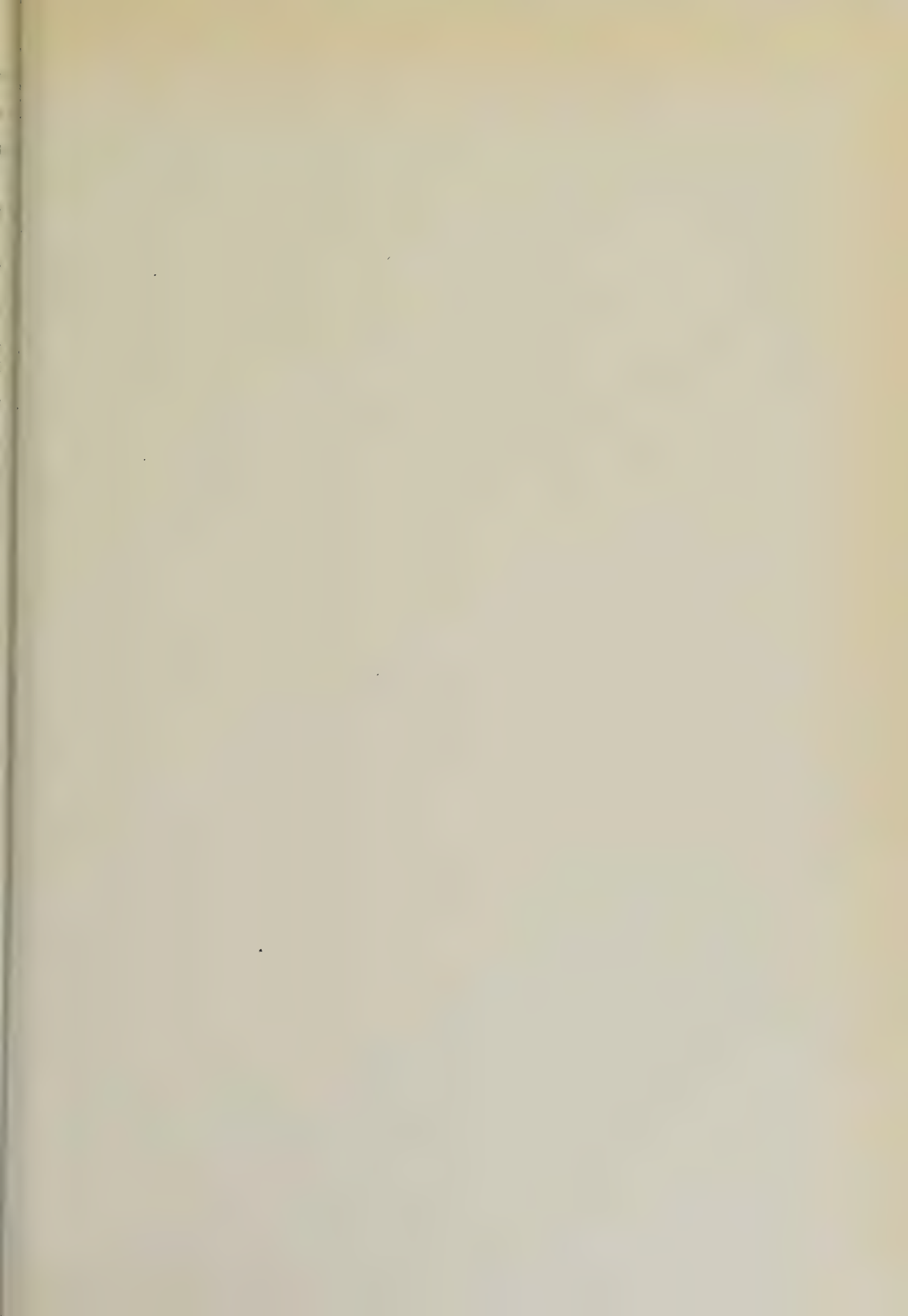
I wish to express my thanks to Prof. W. E. Adams for suggesting the subject of this investigation and particularly for his invaluable advice and criticism in the preparation of the text. I wish, too, to thank Mr J. G. Howard for all his help during the investigation, and Miss M. E. Ogilvie, particularly for the lettering of the illustrations. My thanks are also due to Prof. A. K. McIntyre, of the Physiology Department of the University of Otago, for his helpful suggestions; to Dr Takashi Ito, of Nagoya University, Japan, for summaries in English of Mishima's papers and for sending me Ishida's paper; and to Prof. L. R. Richardson, of Victoria University College, Wellington, and Dr G. Eloff, of the University of the Orange Free State, for their helpful correspondence.

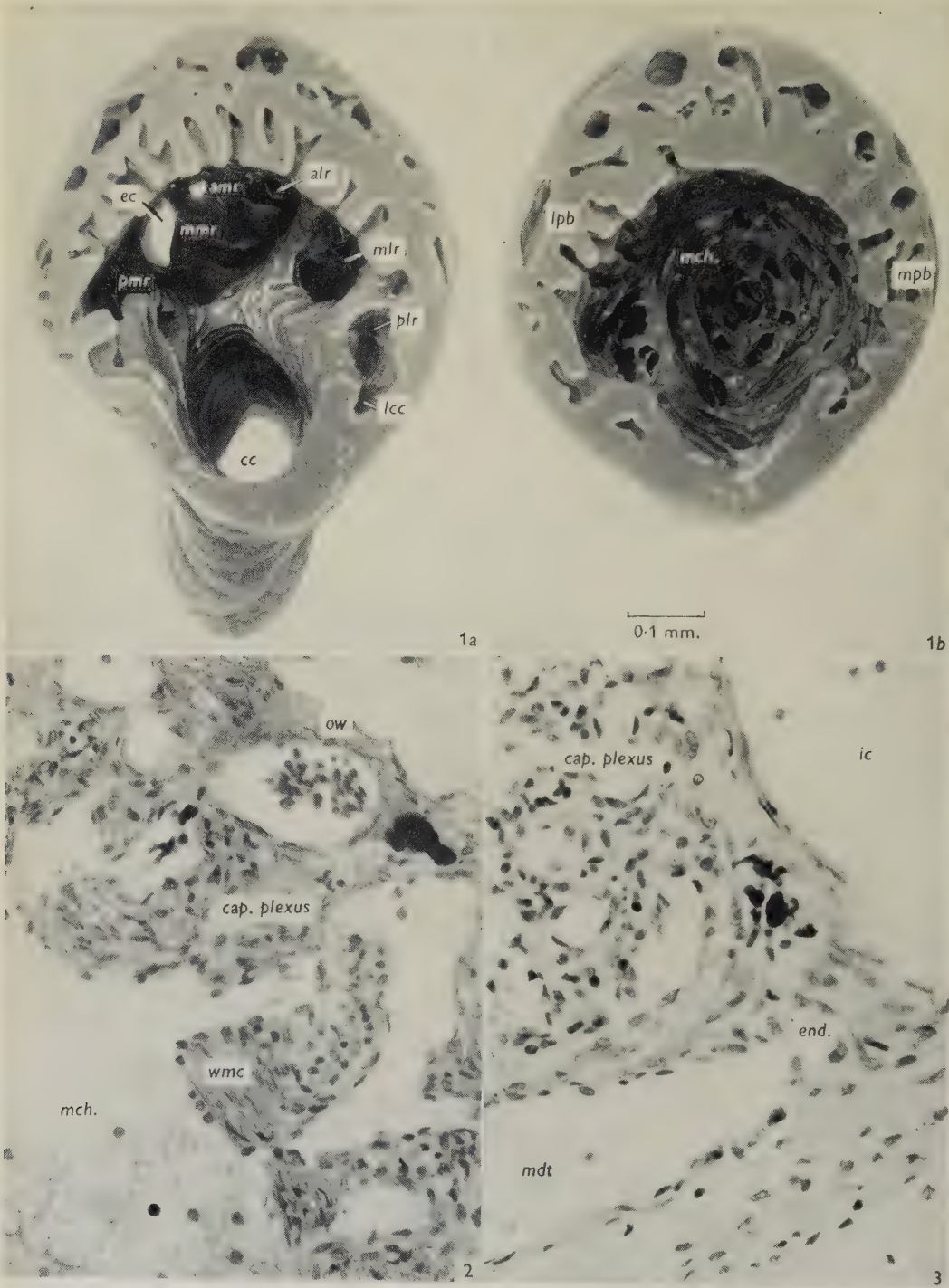
## REFERENCES

- ASK-UPMARK, E. (1935). *The Carotid Sinus and the Cerebral Circulation, an Anatomical, Experimental, and Clinical Investigation* 374 pp. Lund: Berlingska Boktryckeriet.
- BOAS, E. V. (1882). Ueber den Conus arteriosus und die Arterienbogen der Amphibien. *Morph. Jb.* 7, 488–572. (Cited by Pischinger.)
- BOAS, E. V. (1883). Beiträge zur Angiologie der Amphibien. *Morph. Jb.* 8, 169–187. (Cited by Pischinger.)



- DE BOISSEZON, P. (1939). Le labyrinthe carotidien de la grenouille rousse adulte. *Bull. Soc. Hist. nat. Toulouse*, **73**, 145–152.
- BREHM, A. (1912). *Tierleben. Allgemeine Kunde des Tierreichs. IV. Die Lurche und Kriechtiere.* (Cited by Pischinger with no details.)
- BRÜCKE, E. (1852). Beiträge zur vergleichenden Anatomie und Physiologie des Gefäßsystems der Amphibien. *Denkschr. Akad. Wiss. Wien*, **3**, 335–367. (Cited by Zimmermann.)
- CHOWDHARY, D. S. (1951). The carotid labyrinth of *Rana tigrina*. *Nature, Lond.*, **167**, 1074.
- ECKER, A. & WIEDERSHEIM, R. (1881). *Die Anatomie des Frosches.* Zweite Abtheilung, 115 pp. Brunswick: Vieweg und Sohn.
- ELOFF, G. (1935). Carotid gland of the South African bullfrog. *Nature, Lond.*, **136**, 108.
- EVANS, C. L. (1949). *Starling's Principles of Human Physiology*, 10th ed. 1193 pp. London: J. & A. Churchill Ltd.
- FOXON, G. E. H. & WALLS, E. W. (1947). The radiographic demonstration of the mode of action of the heart of the frog. *J. Anat., Lond.*, **81**, 111–117.
- FRANCIS, E. T. B. (1934). *The Anatomy of the Salamander*, 381 pp. London: Oxford University Press.
- GRANEL, F. (1927). La pseudobranchie des poissons. *Arch. Anat. micr.* **23**, 175–317. (Cited by de Boissezon.)
- HOLLINSHEAD, W. H. (1943). A cytological study of the carotid body of the cat. *Amer. J. Anat.* **73**, 185–213.
- HOLLINSHEAD, W. H. (1945). Effects of anoxia upon carotid body morphology. *Anat. Rec.* **92**, 255–261.
- HUSCHKE, E. (1831). Ueber die Carotidendrüse einiger Amphibien. *Z. Physiol.* **4**, 113–118. (Cited by Pischinger.)
- HYRTL, J. (1838). Beobachtungen aus dem Gebiete der vergleichenden Gefäßlehre, III. *Med. Jb. öst. Staates*, **25**. (Cited by Pischinger.)
- ISHIDA, S. (1954). So-called carotic body of the Amphibia. *Igaku Kenkyuu*, **24**, 1024–1050. (In Japanese. See acknowledgements.)
- KUNO, Y. & VON BRÜCKE, E. T. (1914). Der funktionelle Nachweis des Nervus depressor beim Frosch. *Pflüg. Arch. ges. Physiol.* **157**, 117–135. (Cited by Meyer.)
- MARSHALL, A. M. (1893). *Vertebrate Embryology*, 640 pp. London: Smith, Elder and Co.
- MAURER, F. (1888). Schilddrüse, Thymus und Kiemenreste der Amphibien. *Morph. Jb.* **13**, 296–382. (Cited by Pischinger.)
- MAURER, F. (1899). Die Schlundspalten-Derivate von Echidna. *Anat. Anz., Ergänzungsh.*, **16**, 88–101. (Cited by Pischinger.)
- MEYER, F. (1927). Versuche über Blutdruckzügler beim Frosch. *Pflüg. Arch. ges. Physiol.* **215**, 545–552.
- MISHIMA, D. (1944). On the development of the carotid gland of Anurans: I. In *Rhacophorus arborea* Schlegelii. II. In *Bufo formosus*. *Kaibo Z.* **22**, 339–347, 399–408. (In Japanese. Abstract only available; see acknowledgements.)
- NEIL, E., STRÖM, L. & ZOTTERMAN, Y. (1950). Action potential studies of afferent fibres in the IXth and Xth cranial nerves of the frog. *Acta physiol. scand.* **20**, 338–350.
- NOBLE, G. K. (1931). *The Biology of the Amphibia*, 577 pp. New York: McGraw-Hill Book Co. Inc.
- PALME, F. (1934). Die Paraganglien über dem Herzen und im Endigungsgebiet des Nervus depressor. *Z. mikr.-anat. Forsch.* **36**, 391–420.
- PISCHINGER, A. (1934). Über die Entwicklung und das Wesen des Carotislabyrinths bei Anuren. *Z. Anat. EntwGesch.* **103**, 45–52.
- ROMEIS, B. (1926). Morphologische und experimentelle Studien über die Epithelkörper der Amphibien. *Z. Anat. EntwGesch.* **80**, 547–578.
- SABATIER, A. (1873). *Études sur le cœur et la circulation centrale dans la série des vertébrés. Anatomie et physiologie comparées. Philosophie naturelle*, 462 pp. Montpellier: C. Coulet. (Cited by Pischinger.)
- SCHOLZ, J. (1933). Morphologische Untersuchungen über die Epithelkörper der Urodelen. *Z. mikr.-anat. Forsch.* **34**, 159–200.
- STANNIUS, H. (1846). *Lehrbuch der vergleichenden Anatomie der Wirbelthiere*, 482 pp. Berlin: Viet and Co. (Cited by Pischinger.)
- VANDERVAEL, F. (1933). Recherches sur le mécanisme de la circulation du sang dans le cœur des Amphibiens anoures. *Arch. Biol., Paris*, **44**, 577–606. (Cited by Foxon and Walls.)
- ZIMMERMANN, W. (1887). Über die Carotidendrüse von *Rana esculenta*, 37 pp. Inaug. Diss., Berlin.





CARMAN—AMPHIBIAN CAROTID LABYRINTH

(Facing p. 525)



## LIST OF ABBREVIATIONS

<i>a</i>	aorta	<i>ldt</i>	lateral distal trunk
<i>abw</i>	afferent branchial vessel	<i>ln</i>	laryngeal nerve
<i>ah</i>	anterior horn of hyoid bone	<i>lpb</i>	most lateral proximal branch
<i>alr</i>	anterior lateral ramus	<i>lpt</i>	lateral proximal trunk
<i>amr</i>	anterior medial ramus	<i>lr</i>	lateral root of external carotid
<i>ant. lat. ram. (ec)</i>	anterior lateral ramus of external carotid	<i>m</i>	mandible
<i>ax</i>	axillary space	<i>mcc</i>	medial collateral channel
<i>b</i>	floor of buccal cavity	<i>mch.</i>	main chamber
<i>cap. lp.</i>	capillary loop	<i>mcv</i>	musculo-cutaneous vein
<i>cap. plexus</i>	capillary plexus	<i>mdt</i>	medial distal trunk
<i>cc</i>	common carotid	<i>midd. lat. ram. (ec)</i>	middle lateral ramus of external carotid
<i>cch.</i>	communicating channel between roots of external carotid	<i>mlr</i>	middle lateral ramus
<i>cl</i>	carotid labyrinth	<i>mmb</i>	middle medial distal branch
<i>comm. car.</i>	common carotid	<i>mmr</i>	middle medial ramus
<i>comm. ch.</i>	communicating channel (in larva)	<i>mpb</i>	most medial proximal branch
<i>d</i>	deltoid	<i>mpt</i>	medial proximal trunk
<i>da</i>	dorsal aorta	<i>mr</i>	medial root of external carotid
<i>dmb</i>	dorsal medial distal branch	<i>o</i>	osternum
<i>eb</i>	epithelial bodies	<i>oa</i>	obliquus abdominis
<i>ebv</i>	efferent branchial vessel	<i>oh</i>	omohyoid
<i>ec</i>	external carotid	<i>ow</i>	outer wall of labyrinth
<i>ejv</i>	external jugular vein	<i>p</i>	pericardium
<i>end.</i>	endothelium	<i>pa</i>	pars abdominalis of m. pectoralis
<i>ext. car., lat. root</i>	lateral root of external carotid	<i>pc</i>	pulmo-cutaneous artery
<i>f</i>	fat body	<i>plr</i>	posterior lateral ramus
<i>gh</i>	geniohyoid	<i>pmr</i>	posterior medial ramus
<i>gn</i>	glossopharyngeal nerve	<i>post. lat. ram. (ec)</i>	posterior lateral ramus of external carotid
<i>hg</i>	hyoglossus	<i>ps</i>	pars sternalis of m. pectoralis
<i>hn</i>	hypoglossal nerve	<i>r</i>	raphe
<i>ic</i>	internal carotid	<i>ra</i>	rectus abdominis
<i>ima</i>	intermandibularis anterior	<i>scv</i>	subclavian vein
<i>imp</i>	intermandibularis posterior	<i>sh</i>	sternohyoid
<i>int. car.</i>	internal carotid	<i>sp</i>	septum pectoralis
<i>iv</i>	innominate vein	<i>sr</i>	sternoradialis
<i>jb</i>	jugular body	<i>ssm</i>	septum submaxillaris
<i>lat. dist. trunk</i>	lateral distal trunk of internal carotid	<i>svc</i>	superior vena cava
<i>lat. prox. trunk</i>	lateral proximal trunk of internal carotid	<i>ta</i>	truncus arteriosus
<i>lcc</i>	lateral collateral channel	<i>vmb</i>	ventral medial distal branch
		<i>wmc</i>	wall of main chamber
		<i>x</i>	xiphisternum

## EXPLANATION OF PLATE

Figs. 1*a, b*: a wax-plate reconstruction of a right carotid labyrinth (model A) shown in two approximately equal halves; anterior is above and the lateral aspects are adjacent. The proximal (ventral) half (*a*) shows the openings of the six rami of the external carotid arranged in a semicircle anterior to the terminal opening of the common carotid; note that these openings are all situated out of the direct line of blood flow into the main chamber. The distal (dorsal) half (*b*) shows numerous afferent vessels leading into the capillary plexus and also the tributaries of the internal carotid running in the periphery of the anterior half of the organ.

Fig. 2. A section of the anterior portion of the carotid labyrinth showing the markedly fibrous nature of the wall of the main chamber (*wmc*, below) and of the outer wall of the organ (*ow*, above), and also the densely nucleated and very fine connective tissue of the intervening capillary plexus (*cap. plexus*). Note the typical clump of melanophores (upper right). H.-E.,  $\times 370$ .

Fig. 3. A section of the dorsal part of the carotid labyrinth showing a typical portion of the capillary plexus, the medial distal trunk (*mdt*) and part of the internal carotid (*ic*). Note the thin fibrous walls of the main channels, the dense nucleation of the stroma of the capillary plexus (*cap. plexus*), and in particular the portion of endothelium (*end.*) seen face on; it is this occurrence, together with superimposed blood cells, which adds to the complexity of the nuclear pattern in the capillary plexus. H.-E.,  $\times 410$ .

## THE MORPHOLOGY OF THE STERNOMASTOID AND TRAPEZIUS MUSCLES

BY J. MCKENZIE

*Department of Anatomy, University of Aberdeen*

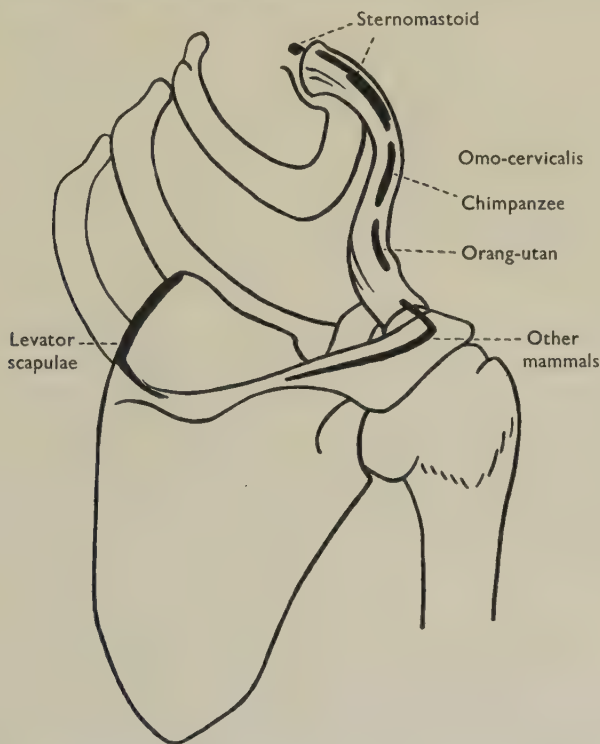
The cleidomastoid portion of the human sternomastoid lying deep to or pierced by the accessory nerve runs oblique to the rest of the muscle. Why should it be so different? Why is there so much doubt in the text-books and variance in the literature on the question of whether the cervical nerves carry efferent fibres to the sternomastoid and trapezius muscles? This investigation attempts to answer these questions.

It is well known (e.g. Streissler, 1900) that the sternomastoid muscle is not composed of the same number of parts in all mammals, and I considered it an important feature of its composition in man that the accessory nerve pierced or lay superficial to this deep (cleidomastoid) portion. The literature, however, dealing with comparative myology is chaotic in regard to the terminology and homology of this muscle; it is impossible to trace the features of the sternomastoid throughout the mammals merely by reference to published work.

Cuvier (1849) illustrates the muscle in a large series of animals but gives no indication of its relationship to the accessory nerve; a narrow sternomastoid is shown in a ventral view of the neck of the pig but is omitted in the lateral view. In a dissection to find how the accessory nerve is related to the muscle, I discovered a stout, strap-like muscle pierced by the nerve and easily divided into a deep and superficial portion, although it differed from the human muscle in that the deep portion was attached inferiorly to the manubrium. Streissler (1900) describes the sternomastoid and trapezius in a series of mammals; in the mole, he found both a superficial and a deep sternomastoid portion in the muscle as well as a part corresponding to a cleido-occipitalis while Cuvier figures only a single sternomastoid and a cleidomastoid part. This cleidomastoid part is shown on the same plane as the sternomastoid portion and although bearing the same name as a part of the human muscle it is, from the illustration, obviously not comparable with it. In a dissection of a mole and in serial sections of a mole embryo, I find that the whole sternomastoid muscle passes superficial to the accessory nerve with no sign of subdivision into superficial or deep portions. Streissler, dealing with the rabbit, recognizes in Krause's work (1884) the mistake in calling an obvious cleido-occipital portion the cleidomastoid, and states (quite correctly) that there are no deep fibres in the sternomastoid muscle, but after describing carefully the attachments of the basihumeralis (omocervicalis), a muscle deep to and quite distinct from the sternomastoid-trapezius group, he labels it, quite wrongly (*clm.*) in his illustration. I take this to mean cleidomastoid as did Edgeworth (1935), who, however, reproduced (rather poorly) Streissler's illustration, giving the muscle the full title of cleidomastoid in spite of Streissler's diagram, in its original form, showing very clearly that the muscle had no connexion whatsoever

with the mastoid process. After studying the matter very carefully I reluctantly decided that Edgeworth uses the names sterno- and cleido-mastoid and sterno- and cleido-occipitalis too loosely to have any significance.

In view of these inconsistencies in the literature, dissections were made in a series of animals to determine the presence or absence of 'deep' fibres in the sternomastoid muscle. During these dissections the enormous variability and plasticity of the omocervicalis (or omotransversarius) suggested a close connexion between this muscle and the deep head of the sternomastoid; if the attachments of the omocervicalis to the shoulder girdle can vary from the lateral end of the spine of the scapula, as in most mammals, round on to the acromion, lateral end of clavicle or even the middle



Text-fig. 1. The variations in the attachments of the omocervicalis to the shoulder girdle.

of the clavicle (Text-fig. 1), and at the same time have its upper (or anterior) attachment migrating to the base of the skull, as in the rabbit, could it not be, then, that the deep portion of the sternomastoid muscle represents an omocervicalis or a part of that muscle?

Serial sections of 13-day and 14-day-old rabbit embryos, a 12 mm. pig embryo and a 9 mm. human embryo were examined. The adult rabbit sternomastoid has no part lying deep to the accessory nerve, whereas a deep portion of the muscle exists both in the pig and in the human embryo.

In the 13-day-old rabbit embryo the upper end of the sternomastoid-trapezius premuscle mass lies in front of the anterior cardinal vein. As this mass is traced



caudally in serial sections, it moves dorsally between the vein and the surface, and at its lower end it lies near the myotome group of muscles. The accessory nerve leaves the vagus-accessory group, curls round the anterior surface of the vein and dives into the deep surface of the muscle mass. In the 14-day-old embryo (Pl. 1, figs. 1-4) the conditons are clearer; the upper end of the mass is antero-lateral to the vein, bifurcating as it curves outwards and backwards and finally touching the myotome derivatives with its posterior division (i.e. trapezius). The accessory nerve lies deep to the sternomastoid muscle and ends in the trapezius. The omocervicalis, a discrete mass derived from the myotomes, lies between the cranial half of the sternomastoid-trapezius mass and the myotomes.

In the 12 mm. pig embryo, the cranial end of the sternomastoid-trapezius mass is lateral to the anterior cardinal vein, but is not clearly defined from the myotomes posteriorly—there is a definite mesenchymal condensation linking the two masses. This connexion is lost as the sternomastoid-trapezius mass moves ventrally alongside the vein to meet the accessory nerve which curls round the front of the vein and plunges immediately into the premuscle mass. At the lower end the posterior border of the trapezius cannot be clearly demarcated from the myotome musculature. The omocervicalis projects from the myotomes just behind the condensation linking sternomastoid and myotomes and in front of the myotome projection in contact with the trapezius.

In the 9 mm. human embryo (Plate 1, figs. 5-8) the upper end of the sternomastoid-trapezius mass, lying lateral to the anterior cardinal vein, is more like an extension of the myotomes. When traced along its length, it shakes itself free, meets the accessory nerve antero-lateral to the vein and thereafter divides into the two muscles. The nerve passes through the sternomastoid mass to lie on the deep surface of the trapezius descending closely applied to the myotome projection which gives rise to the serratus anterior-levator scapulae-rhomboid group of muscles.

#### DISCUSSION

Straus & Howell (1936) carried out an extensive review of the literature when investigating the spinal accessory nerve and its musculature, and concluded that this nerve 'exhibits a strong phylogenetic inclination to lose its sensory cells by their migration onto the dorsal roots of adjacent cervical nerves. The motor fibres appear to be somewhat more conservative but eventually they also tend to follow this new and shorter spinal pathway...'. Streeter (1905), supported by Fahmy (1927), had already shown that during development there are more sensory cells in the accessory nerve of the foetus than in the adult. But the problem of whether the sternomastoid muscle does receive efferent fibres or not through the upper cervical nerves has not been satisfactorily answered. As early as 1891, Chauveau attempted to solve the problem by section of the cervical nerves entering the sternomastoid of the horse followed by stimulation of the peripheral ends. He obtained no reaction in the muscle and concluded that there were no motor fibres in these nerves as they enter the muscle. Subsequent efforts to confirm or refute his conclusion in the horse, cow, dog, monkey and chimpanzee have given inconsistent results. Strauss & Howell (1936) themselves carried out the experiment in twenty-three dogs; stimulation of the

peripheral ends of the nerves resulted in muscular action of the sternomastoid in more than half the cases and of the trapezius in considerably less than half the cases. Their admission to being uncertain of finding and stimulating all cervical filaments in each experiment infers their belief that there should have been a reaction in every case. These authors based their conclusions that the motor nerves were taking the shorter spinal course, partly on these observations, partly because of the 'phylogenetic and ontogenetic inclination' of the sensory fibres to travel with cervical nerves and partly because they believed that the spinal accessory nerve tends to disappear as a gross entity in long-necked mammals. Zuckerman & Kiss (1932), however, found a spinal accessory nerve in two specimens of the giraffe which they dissected.

Without denying the possibility that the motor nerve fibres to the sternomastoid and trapezius may sometimes take the shorter route, I wonder why the trapezius, requiring a much longer path for its nerves than the sternomastoid, should have an apparently smaller cervical efferent supply than the sternomastoid, if we are to judge by the experiments of Straus and Howell. I think there is a more obvious explanation of the presence of efferent fibres to the sternomastoid and trapezius in the cervical nerves and an explanation of the inconsistent results of the various experiments. Where part of the adult sternomastoid muscle lies deep to or is pierced by the accessory nerve (e.g. man, pig), the developing premuscle mass lies lateral to the anterior cardinal vein, is linked with the myotomes at its upper end and moves ventrally to meet the nerve. Where there is no deep portion to the sternomastoid muscle (e.g. rabbit) the upper end of the muscle is anterior to the vein, ready to receive the accessory nerve, in its earliest recognizable stages and has no connexion with the myotomes. In spite of Lewis's (1912) assertion that there is no myotome contribution to the sternomastoid and trapezius and that it is entirely branchial in origin, I think this comparison of human and pig with rabbit embryos is significant in showing a difference in the development of the muscle depending on whether a deep portion of the muscle exists in the adult, i.e. where part of the sternomastoid muscle lies deep to or is pierced by the accessory nerve then the muscle has been derived in part from myotome material and will consequently receive motor fibres through the upper cervical nerves. An explanation of the cervical motor supply to the trapezius is suggested by the proximity of the trapezius to the myotomes and in particular to the serratus anterior-levator scapulae-rhomboid group—a feature common to both rabbit and human embryos.

Accordingly, when it is appreciated that there is no adequate description of the composition of the sternomastoid muscle in the different animals and that the deep part of this muscle is not always present, then the variable results from experimental work on the cervical nerves supplying the muscle are intelligible. For example the accessory nerve in the horse is deep to the muscle (sterno-maxillaris) which Chauveau (1893) regards as corresponding to the sterno-mastoideus of man. Yet the mastoido-humeralis lateral to the sterno-maxillaris also receives a supply from the accessory nerve and is pierced by it. Concerning that part of the mastoido-humeralis lying superficial to the nerve, there is no doubt that it belongs to the sternomastoid-trapezius group but its deeper portion is suspiciously like an omocervicalis. What, in fact, did Chauveau (1891) accept as the sternomastoid in his experiment? No deep

part exists in the monkey (macaque), whereas it is well defined in the chimpanzee, cat and dog; it was to be expected, then, in Straus & Howell's work on the dog, that cervical nerves entering the muscle should carry efferent fibres.

Giebel (1874–1900) suggested that the omocervicalis is an extension of the levator scapulae-rhomboid sheet of muscles, and when the omocervicalis in the rabbit embryo and the link between the sternomastoid and myotomes in the human embryo are compared, it seems reasonable to accept them as being homologous—in other words, the omocervicalis is represented within the human sternomastoid. Nor is it difficult to understand how there can be both a deep part to the sternomastoid and an omocervicalis—there must be a potential sheet of muscle, draped from the upper cervical transverse processes and base of skull, attached distally to the spine of scapula, acromion and clavicle and providing muscle or muscles as required.

In view of the interchange of muscle fibres between the superficial and deep parts of the adult sternomastoid and the absence of subdivision in the embryonic pre-muscle mass giving rise to the muscle, the myotome contribution is probably distributed (although not evenly) throughout the whole sternomastoid, but this aspect of the problem requires further investigation by degeneration experiments.

#### CONCLUSIONS

In the comparative anatomy of the sternomastoid muscle there are obvious errors in nomenclature in work of accepted standing, even where the author is merely citing a contribution of a previous worker.

The sternomastoid muscle as it occurs throughout the mammals requires more careful description, especially in regard to its relationship to the accessory nerve, for this is the key to the problem of its efferent nerve supply.

The sternomastoid in man and in the pig, where there is a portion of the muscle lying deep to or pierced by the accessory nerve, contains muscle fibres of myotomic origin. These fibres represent in whole or in part the omocervicalis which is present in most other mammals and is derived from the levator scapulae-rhomboid group of muscles.

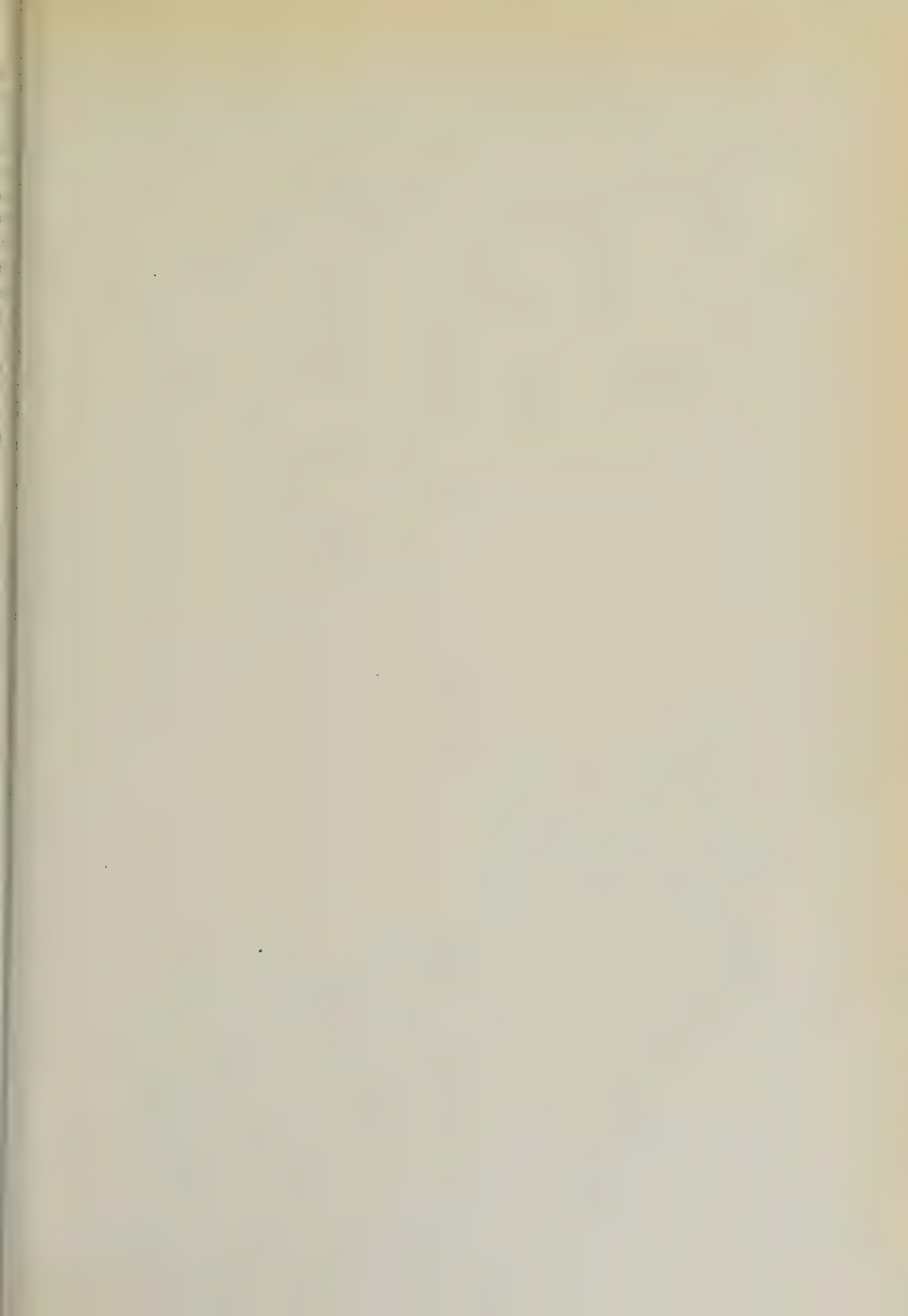
The sternomastoid of the rabbit with no deep portion contains no fibres of myotomic origin.

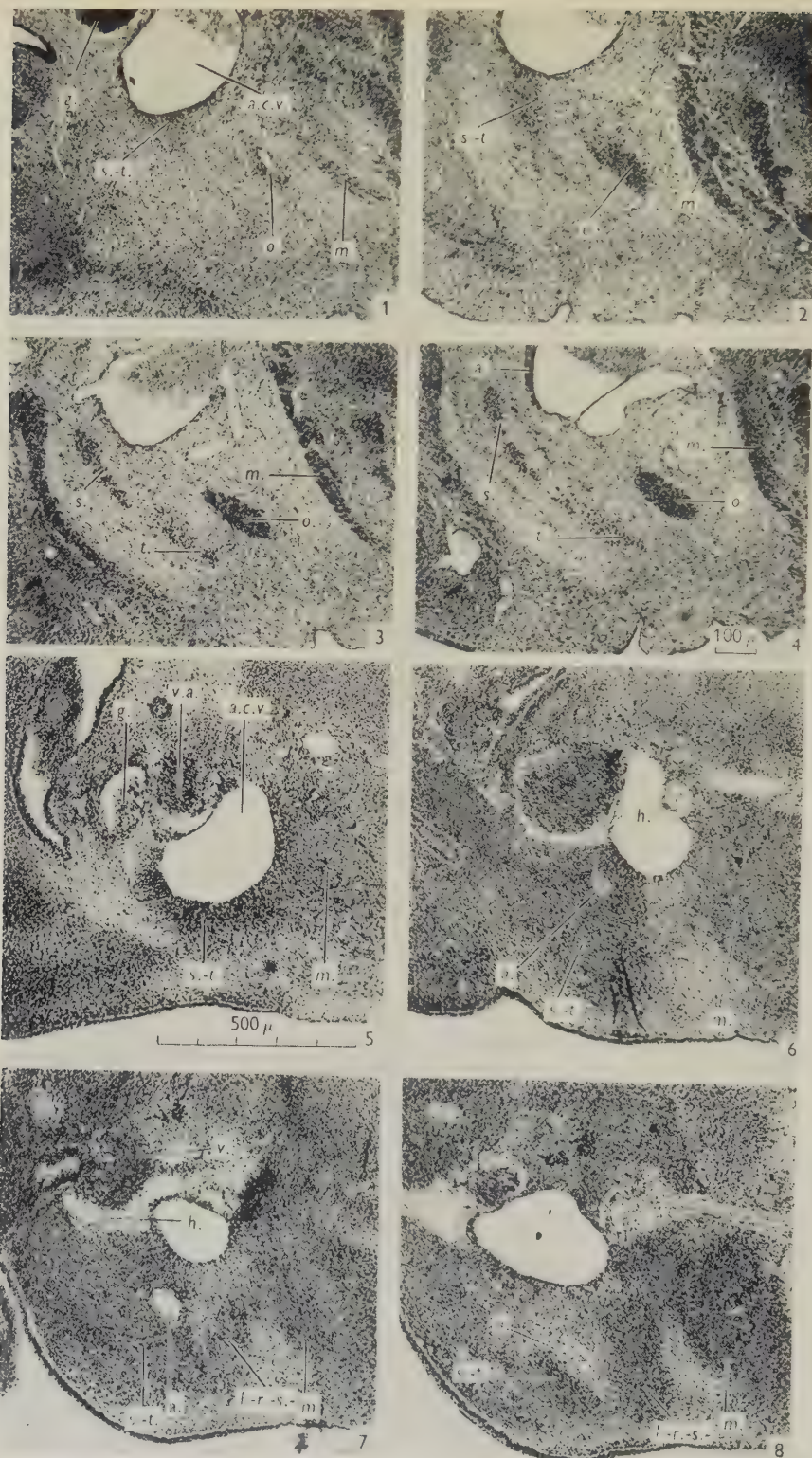
The trapezius in man, pig and rabbit probably derives muscle fibres of myotomic origin and receives cervical nerve efferents as a result of its close association during development with the muscle masses arising from myotomes.

This composite nature of the sternomastoid in man and in those animals with a deep portion to the muscle, e.g. dog, pig and chimpanzee, explains the presence of efferent fibres in the branches of the cervical nerves entering it. Where there is no deep part to the sternomastoid muscle, efferent fibres in these nerves cannot be expected to occur.

I am indebted to Prof. R. D. Lockhart for his encouragement, helpful advice and criticism throughout this work, to Prof. J. C. Brash for permission to dissect the primate specimens in his department, and to Mr A. Cain for the preparation of the microphotographs.







# REFERENCES

- CHAUVEAU, A. (1891). On the sensorimotor nerve-circuit of muscles. *Brain*, **14**, 145-178.
- CHAUVEAU, A. (1893). *The Comparative Anatomy of the Domesticated Animals*, 2nd ed. p. 255. New York: D. Appleton and Co.
- CUVIER, G. (1849). *Anatomie Comparée*. Paris: Chez Dusacq, Libraire Agricole de la Maison Rustique.
- EDGEWORTH, F. H. (1935). *The Cranial Muscles of the Vertebrates*. Cambridge University Press.
- FAHMY, N. (1927). A note on the intracranial and extracranial parts of the ninth, tenth and eleventh nerves. *J. Anat., Lond.*, **61**, 298-301.
- GIEBEL, C. G. (1874-1900). In Bronn's *Klassen und Ordnungen des Thier-reichs (Säugethiere)*, Band 1, p. 731. Leipzig: C. F. Winter'sche Verlagshandlung.
- KRAUSE, W. (1884). *Die Anatomie des Kaninchens*, 2nd ed. p. 145. Leipzig: Verlag von Wilhelm Englemann.
- LEWIS, W. H. (1912). The development of the muscular system. In Kiebel and Mall, *Manual of Human Embryology*, vol. 1, pp. 454-522. Philadelphia, U.S.A.: J. B. Lippincott Co.
- STRAUS, W. L. & HOWELL, A. B. (1936). The spinal accessory nerve and its musculature. *Quart. Rev. Biol.* **11**, 387-405.
- STREETER, G. L. (1905). The development of the cranial and spinal nerves in the occipital region of the human embryo. *Amer. J. Anat.* **4**, 83-116.
- STREISSLER, E. (1900). Zur vergleichenden Anatomie des M. cucullaris und M. sternocleidomastoideus. *Arch. Anat. Physiol., Lpz.* (Anat. Abt.), pp. 335-365.
- ZUCKERMAN, S. & KISS, F. (1932). The spinal accessory nerve of the giraffe. *Proc. zool. Soc. Lond.*, pp. 767-769.

# EXPLANATION OF PLATE

## Key to figures

<i>a.</i>	accessory nerve	<i>o.</i>	omocervicalis
<i>a.c.v.</i>	anterior cardinal vein	<i>s.</i>	sternomastoid
<i>g.</i>	glossopharyngeal nerve	<i>s.-t.</i>	sternomastoid-trapezius premuscle mass
<i>h.</i>	hypoglossal nerve	<i>t.</i>	trapezius
<i>l.-r.-s.</i>	levator scapulae-rhomboid-serratus anterior muscle group	<i>v.a.</i>	vagus and accessory nerves
<i>m.</i>	myotome	<i>v.</i>	vagus nerve

Figs. 1-4. Transverse sections of a 14-day-old rabbit embryo, from cranial to caudal end of the sternomastoid-trapezius premuscle mass.

Figs. 5-8. Transverse sections of a 9 mm. human embryo from cranial to caudal end of the sternomastoid-trapezius premuscle mass.



# OBSERVATIONS ON ENDOCRANIAL CASTS OF RECENT AND FOSSIL CETACEANS

BY A. S. BREATHNACH

*Department of Anatomy, St Mary's Hospital Medical School, London, W. 2*

## INTRODUCTION

The problems of the ancestry of the modern whales (Odontoceti and Mysticeti) and especially that of their relationship to the extinct cetaceans of the Eocene and Oligocene (Archeoceti) as well as the Miocene Squalodonts, is still very much a matter of controversy, largely because of the lack of intermediate types which might serve to bridge the morphological gaps between the three groups. Dart (1923), on the basis of an examination of a series of endocranial casts, concluded that the Archeocetes cannot be regarded as being on the direct line of cetacean evolution because of certain specializations manifest in the brain, viz. enormous hypertrophy of the trigeminal apparatus and cerebellum. This interpretation of the casts and the widespread conclusions concerning the manner of life, probable habitat and relationships of the Archeocetes which are based on it have been given considerable weight by palaeontologists, e.g. Kellogg (1928, 1936), Flynn (1947), and the most recent writer on this subject—Edinger (1955)—after an examination of the actual casts has reached conclusions concerning the form of the brain which do not differ significantly from those arrived at by Dart. If these authors' views be accepted, then the Archeocete brain must be regarded as presenting an arrangement unique among mammals almost to the point of being grotesque. There is no *a priori* reason why this should not be so, but the form of the brain as read by Dart (1923), Edinger (1955), and others from the endocranial cast is so peculiar that (bearing in mind the highly equivocal nature of the information which can be derived from such material) it is difficult to accept it on neurological grounds, unless it can definitely be shown that no other interpretation is possible.

Marples (1949), as a result of a study of two endocranial casts of very doubtful horizon but which he considered probably belonged to Archeocetes, has tentatively suggested that the alleged immense cerebellum of the Archeocetes might be a misinterpretation of a large vascular plexus of a nature similar to the intra-cranial retia mirabilia which are such a characteristic feature of modern cetaceans. It cannot be known for certain whether or not the Archeocetes possessed such structures, although in view of their complete adaptation to an aquatic life it is at least possible, if not probable, that they did, since such structures are common to all living cetaceans. If so, obviously the presence of retia will be reflected in the endocranial cast, making it a very misleading representation of the brain.

It is evident from figures published (e.g. by Gervais, 1871) that the endocranial cast in modern cetaceans, with well-developed retia, presents but a poor caricature of the form of the brain. However, precise information is lacking as to the type and situation of such distortion of the cast (considered as a reasonable replica of

the brain) which might be directly attributed to the presence of retia and other intracranial vascular structures. Accordingly, it was considered of some interest to examine this question more fully in order to determine whether or not, in living cetaceans, the appearances of whose brains are well known, the presence of retia can alter the form and proportions of the brain as reflected in the endocranial cast, in such a manner as to suggest either presence or absence of similar structures in the Archeocetes. This may enable one to assess their role as factors contributing to the alleged bizarre appearance of the 'brain' in this group, and perhaps substantiate Marples's (1949) suggestion from a different viewpoint.

#### MATERIALS AND METHODS

Apart from illustrations of brains and casts published by other authors, and referred to in the text, the following material was examined.

(1) Brain and endocranial cast of foetal fin-whale (*Balaenoptera physalus*) 14 ft. long, of an estimated (using Walmsley's, 1938, curve of growth during gestation) age of 10–11 months.

(2) Brain and endocranial cast of adult common porpoise (*Phocaena phocaena*). These were not from the same specimen.

(3) Endocranial cast of common dolphin (*Delphinus delphis*).

(4) In addition, well fixed and undistorted brains of the adult fin-whale (*Balaenoptera physalus*) and humpback whale (*Megaptera novaeangliae*) were available, and through the kindness of the Museum authorities access was had to the series of Archeocete skulls and endocranial casts housed in the British Museum.

#### OBSERVATIONS

It is not intended here to make a very detailed and point-for-point comparison between casts and brains. The main interest is to determine to what extent the cast reflects the form, arrangement and proportions of the major subdivisions of the brain, and how far its shape is determined by the presence of vascular or other tissues, apart from the brain, within the cranial cavity.

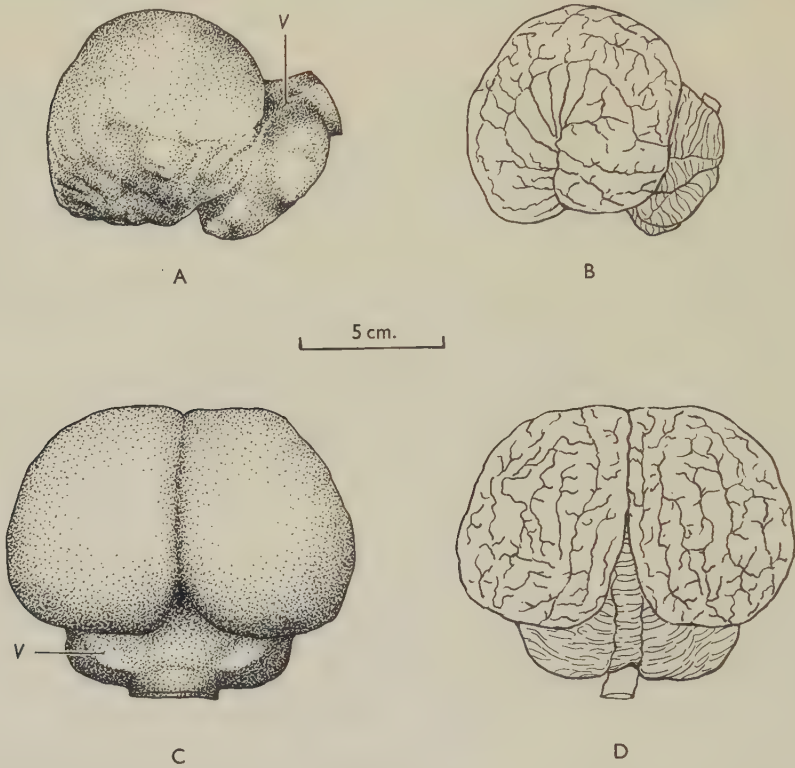
#### *Odontoceti*

It can be seen from Text-fig. 1 that there is a close correspondence between the endocranial cast and the brain in the porpoise. The cast gives a reasonable idea of the brain and of the general proportions and mutual relations of the cerebrum and cerebellum. These remarks apply equally to the endocranial cast of *Delphinus* (not illustrated), and judging from figures of similar casts of other recent Odontocetes such as those of *Cephalorhynchus hectori* and *Pontoporia blainvillii* published by Marples (1949) and Dal Piaz (1905) respectively, this would appear to be a general finding as far as the smaller Odontoceti are concerned.

On closer examination certain differences become apparent. Whereas there is very little distortion of the cerebral hemisphere in the cast and while the approximate position of the lateral fissure can be readily determined, it is evident when one compares the superior view of cast and brain (C and D, Text-fig. 1) that considerably less of the 'cerebellum' is visible from this aspect in the former. This is due to the presence of

retial tissue in relation to the posterior end of the cerebral hemisphere and above the bony tentorium. An exactly comparable state of affairs is evident from Dal Piaz's (1905) photograph of a similar view of the endocranial cast of *Pontoporia*; even less of the cerebellar portion can be seen than in the present instance.

When viewed from above (Text-fig. 1 C, D) the cerebellar portion of the cast can be seen to consist of three portions, two rounded lateral parts and a large elevated central portion continuous with the cast of the foramen magnum. A ridge caused by



Text-fig. 1. Lateral and dorsal views of endocranial cast (A and C) and brain (B and D) of common porpoise (*Phocaena phocaena*). V, cast of blood vessel.

a blood vessel (V) limits this latter portion on either side. Comparison with the corresponding view of the brain shows that these three parts bear very little relation to the actual disposition of the vermis and lateral lobes of the cerebellum.

From the lateral aspect (Text-fig. 1 A, B) it is evident that the antero-posterior diameter of the cerebellar region of the cast is considerably greater than in the brain and, more important (since the two are not from the same specimen), that it is also greater in relation to the corresponding diameter of the cerebral hemisphere. The vertical diameter is also greater in the cast.

A close comparison between the cerebellar region of the cast and the actual cerebellum leads to the conclusion that no information of any value concerning the fissural pattern and morphological subdivisions of the latter can be gained from a

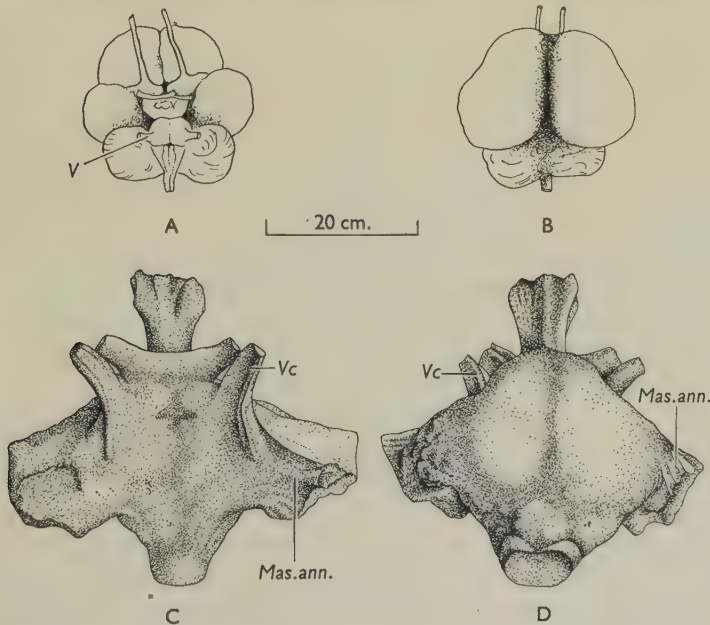


study of the cast. In fact it may be positively misleading. This is only to be expected since the cerebellum is nowhere in direct contact with the skull, and even if it were, the lips of the cerebellar fissures are so close together that one would not expect to find any significant impressions on the inner table.

One might fairly conclude from these observations that considering the amount and distribution of the retial tissue in the smaller Odontoceti, it is surprising how good an impression of the general proportions and shape of the brain can be obtained from the endocranial cast. There is some distortion tending towards enlargement of the posterior pole of the cerebral hemisphere and of the various dimensions of the cerebellum. Details of cerebellar morphology are not reflected in the cast. It is probable that these remarks would not apply in like measure to the larger Odontocetes such as the sperm whale, in which the retial tissue is much more strongly developed.

### *Mysticeti*

Gervais, who worked only with the skeleton, published (1871) some excellent illustrations of endocranial casts from a series of Mysticetes. C and D, Text-fig. 2, are drawn from ventral and dorsal views respectively of Gervais's cast of *Megaptera*,



Text-fig. 2. A and B: outlines of ventral and dorsal aspects respectively of a brain of the hump-back whale (*Megaptera novaeangliae*) drawn from photographs. C and D: corresponding views respectively of endocranial cast of same after Gervais (1871). *Mas. ann.*, masses annexes, *V*, trigeminal nerve; *Vc*, cast of ophthalmic nerve and accompanying rete.

and above them (Text-fig. 2 A, B) are outlines traced from photographs of a brain from the same species in our possession. The figures show in a striking manner how inadequate and distorted an impression of the size and form of the brain can be gained from the endocranial cast of a typical Mysticete.

It will be noted that the form and limits of the cerebrum and cerebellum cannot be inferred from the cast, and that extending laterally on either side from the approximate region of confluence of the two are large masses, the 'masses annexes' of Gervais (1871), which have no counterpart in the brain, and which represent the mass of retial and vascular tissue surrounding the cerebellum and accompanying certain of the cranial nerves on their passage towards exit from the skull. There is a great disparity between the actual size of the trigeminal nerve and the apparent counterpart of the only one of its branches (*Vc*) which can be identified in the cast, due again to the fact that in the living animal the branches of this nerve are accompanied in their intra-cranial course by considerable masses of retial tissue. The actual size of the olfactory peduncles bears little relation to that of the central pedunculated mass which projects from the anterior end of the cast, and which represents the olfactory fossa, of which the peduncles and bulbs occupy only a very small part.

All of these features, with minor differences of degree in different species, are visible in other casts illustrated by Gervais.

#### *Comparison of Mysticete and Archeocete endocranial casts*

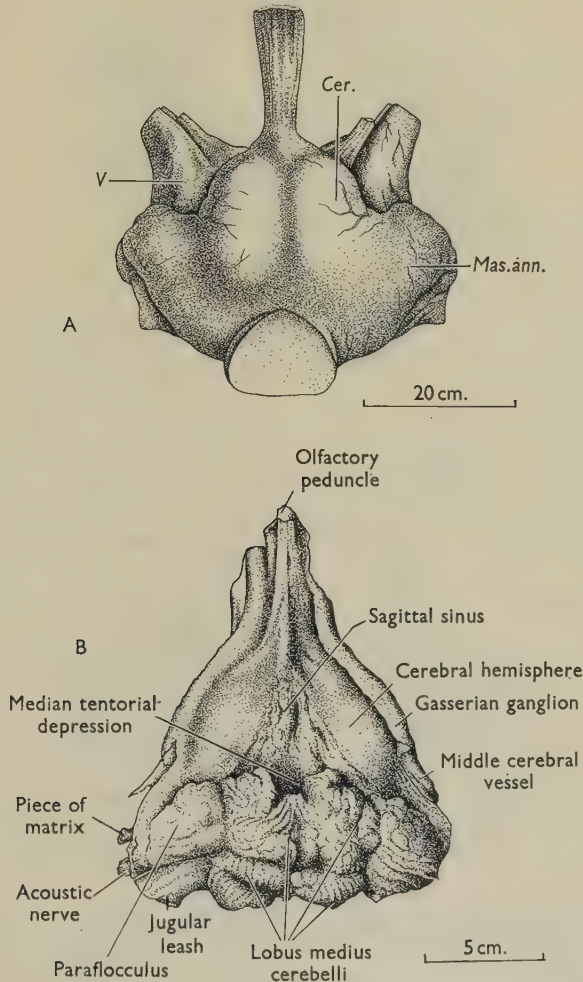
So far as the endocranial casts are concerned it was at once clear (bearing in mind the time factor and the differences in the form of the skull) that the Archeocete resembles the Mysticete far more closely than the Odontocete. Now the two main features which characterize the Archeocete brain, according to Dart (1923) and those who accept his interpretations, are the enormous size of the trigeminal apparatus, and the marked extension of the cerebellum, particularly in a lateral direction. Since, as has been shown in the previous section, the presence of retia can account for a marked exaggeration in the size of the trigeminal nerve and its branches, as well as a marked lateral extension opposite the cerebro-cerebellar junctional area in the endocranial cast of recent cetaceans, it is obviously of interest to compare examples of recent and fossil casts. It is possible that what are appearances of a similar order might have the same underlying causes.

In Text-figs. 3 and 4, dorsal and lateral views respectively of the endocranial casts of two Archeocetes as interpreted by Dart are figured together with a similar view of casts of two modern cetaceans as figured by Gervais (1871).

In each the general position of the cast of the cerebral hemisphere can be made out, as well as a mass which extends in a forward direction alongside it. In the Archeocete this is regarded by Dart as representing an enormous trigeminal ganglion and nerve, while in the recent cetacean we have seen that the structure which occupies a corresponding position, although associated with this nerve, bears little relation to its actual size, and represents mainly the cast of a large amount of retial tissue which accompanies the nerve and its branches.

Directly posterior to this 'trigeminal' mass in both Archeocete and the modern cetaceans, the cast is considerably widened, due in the former, according to Dart, to an enormous hypertrophy of the cerebellum, and of the 'paraflocculus' in particular. The corresponding portion of the cast in modern cetaceans is largely a cast of retial and vascular tissue (masses annexes of Gervais) surrounding the cerebellum and

accompanying the more posterior cranial nerves (Text-fig. 3A). It may be noted in passing that Dart labels as 'medulla oblongata' the cast of the foramen magnum in the Archeocete, and he concluded that this portion of the brain was widened due to hypertrophy of the 'tuberculum quinti' (spinal nucleus and tract of the trigeminal).

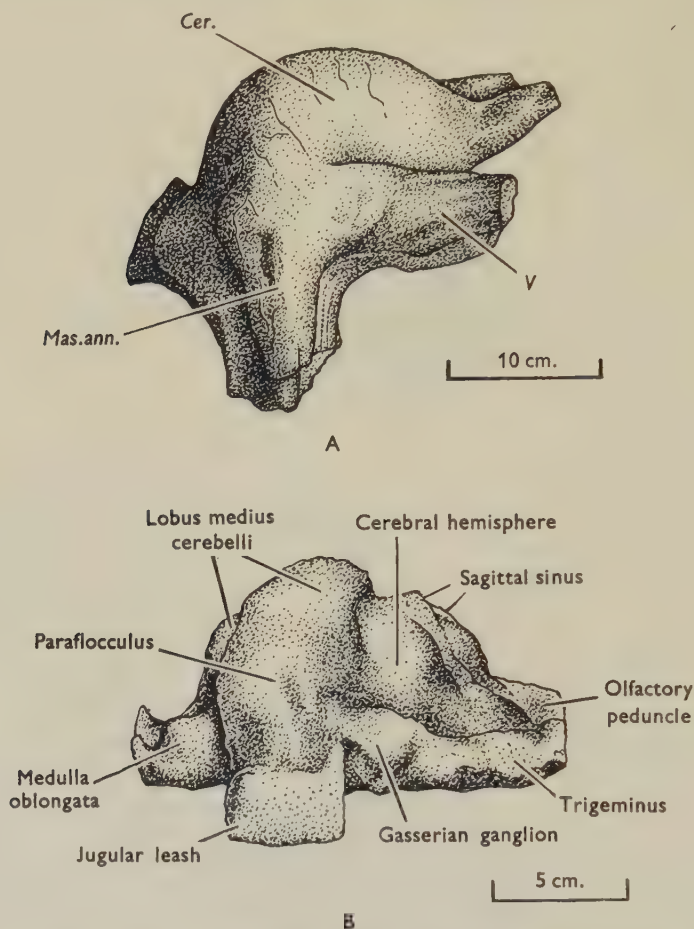


Text-fig. 3. A: dorsal view of endocranial cast of *Balaena mysticetus* after Gervais (1871). *Cer.*, cerebral hemisphere; *Mas. ann.*, masses annexes; *V*, cast of space occupied by retial tissue surrounding trigeminal nerve. B: dorsal view of endocranial cast of the Archeocete *Zeuglodon sensitivus* after Dart (1923) brought up to approximately the same overall size as A. Dart's labelling unchanged.

From this brief comparison it is evident that appearances of a similar order to those which are known to be due to the presence of retia in certain modern cetaceans occur in corresponding situations in the endocranial casts of Archeocetes. Since the latter were also cetaceans and fully adapted to an aquatic environment, a strong



suspicion is aroused that they may also have possessed retia and that the features of the casts which have been interpreted as an enormous development of the cerebellum and trigeminal apparatus may be accounted for by this.

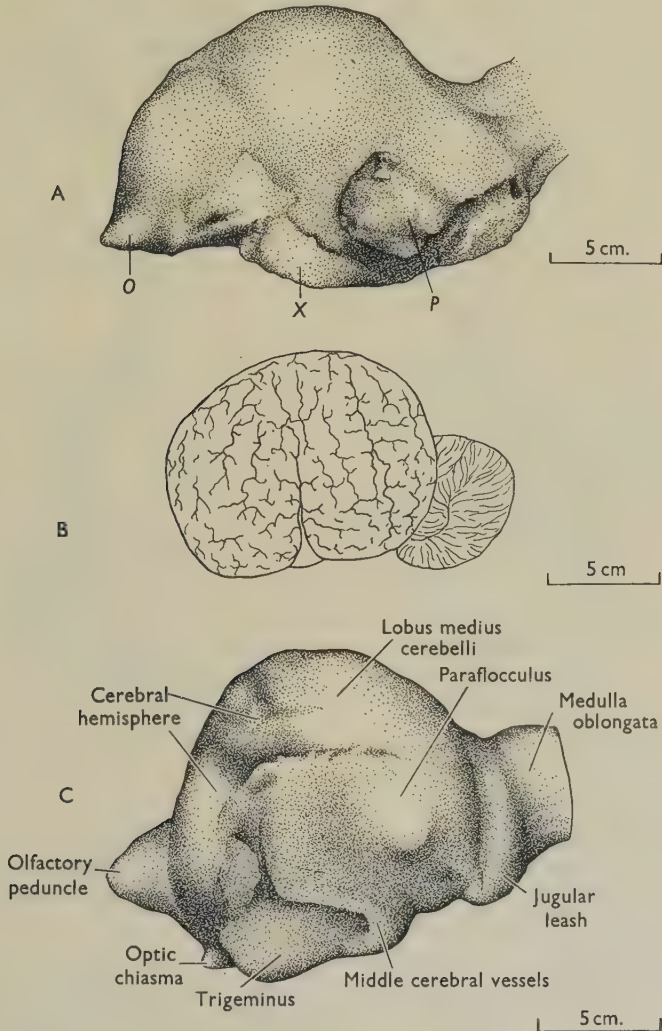


Text-fig. 4. A: lateral view of endocranial cast of *Balaenoptera rostrata* after Gervais (1871). Abbreviations as in Text-fig. 3A. B: lateral view of endocranial cast of the Archeocete *Zeuglodon Osiris* after Dart (1923) brought to approximately the same size as A. Identifications according to Dart.

#### *Comparison of endocranial casts of foetal fin-whale and Prosqualodon*

Lateral and ventral views of the endocranial cast of a foetal fin-whale are illustrated in Text-figs. 5A and 6B. Partly owing to the lesser development of the retia in foetal stages it is not so extraordinary in shape as the adult casts figured by Gervais (1871). Taking this cast by itself it is not difficult to see a general resemblance to a mammalian brain; it could plausibly be suggested that the anterior projection (O) represented a fairly large olfactory bulb, and that the lateral projection from the

posterior end (*P*) was evidence for the presence of a very large flocculus, or para-flocculus, or both. A comparison with an outline of the actual brain from this specimen (Fig. 5B) shows how false any such interpretation would be. The apparent

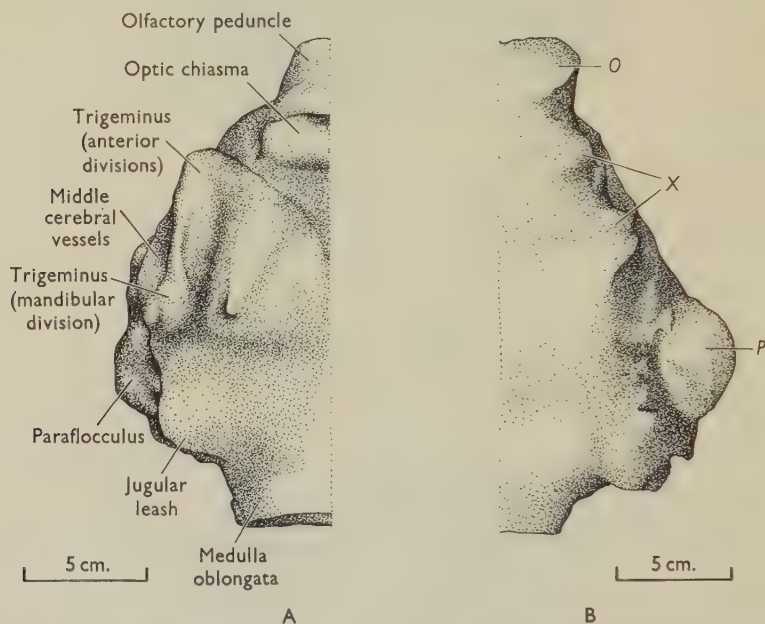


Text-fig. 5. A: lateral aspect of endocranial cast of foetal fin-whale (*Balaenoptera physalus*). The letters indicate structures referred to in the text (p. 538). B: lateral aspect of the brain from the same specimen drawn from a photograph. C: lateral aspect of endocranial cast of *Prosqualodon davidi* Flynn after Dart (1923) brought to approximately the same size as A. Dart's labelling unchanged.

olfactory bulb is the cast of the olfactory fossa (Pl. 1, fig. 1) lying anterior to the brain, and which, as is evident from Pl. 1, fig. 2, bears little relation to the actual size or shape of the olfactory peduncle, while the projection (*P*) in the cast has no counterpart whatsoever in the brain. It is obvious that the presence of the retina and

other endocranial structures apart from the brain has so modified the shape of the cranial cavity that it yields virtually no reliable information about the morphology of the brain, and is in fact seriously misleading if used for this purpose.

The endocranial cast of *Prosqualodon*, illustrated by Dart (1923), is rather similar in shape to that of the foetal fin-whale (see Text-figs. 5C and 6A, outlines traced from Dart's figs. 21 and 18 respectively, orientated similarly to the cast from the foetal fin-whale). One notes a similar precerebral projection which is taken to represent a nipple-like olfactory bulb, from which identification certain far-reaching conclusions were drawn. It is certain that this projection represents the cast of the



Text-fig. 6. A: ventral aspect of right half of endocranial cast of *Prosqualodon davidi* Flynn after Dart (1923) brought to approximately the same size as B. Identifications according to Dart. B: similar view of left half of endocranial cast of foetal fin-whale (*Balaenoptera physalus*). The structures labelled are referred to in the text (p. 540).

'olfactory fossa' figured by Flynn (1947; Text-fig. 4) and there is no evidence that it represents the actual size or manner of attachment of the bulb in *Prosqualodon*. Again, the structure confidently labelled 'paraflocculus' by Dart bears a striking resemblance in shape and position to the structure *P* in the foetal cast, which, as is evident, does not represent any part of the brain but rather the cast of a space occupied by vascular tissue. Finally, there is present in the foetal cast a prominence (*X*, Text-fig. 6B), similar in situation to the 'trigeminus' of Dart, which can again be accounted for by the presence of intra-cranial vascular tissue. One might legitimately conclude from the above that in some respects the skull of *Prosqualodon* is more like the foetal than adult stages of the skull of modern Mysticetes, a conclusion which could be tested by reference to the actual bones. One might also suggest that the similarity between the casts is at least partly due to the presence



of retial tissue within the cranial cavity of both animals, as is certainly the case in the one living to-day. This suggestion must remain a speculation so far as *Prosqualodon* is concerned, but it is far more in line with what one would expect to find within a cetacean skull than the almost incredible hypertrophy of the cerebellum and trigeminal apparatus which Dart's interpretation necessitates. It might, of course, be argued that *Prosqualodon* should be compared with the Odontocete rather than that with a Mysticete cetacean, and again, though less striking, there is some similarity in the casts (compare Text-figs. 1A and 5C). It has already been shown that the Odontocete cast gives a fair but by no means perfect representation of the brain, but this does not help Dart's interpretation. If the similarity means anything it is that the Squalodont brain may have resembled that of a modern Odontocete (Text-fig. 1B) and not that it possessed a cerebellum considerably larger than the cerebral hemisphere, and a trigeminal apparatus of large proportions.

#### DISCUSSION

One may summarize the present findings by stating that the endocranial cast of the smaller Odontocetes gives a reasonable picture of the form of the brain, but with distortion of the cerebellar region, while in the Mysticetes in general, the cast is little less than a poor and misleading caricature. This difference is mainly accounted for by the exuberance of intra-cranial retial tissue in the latter group as compared with the former. The possibility that the Archeocetes and *Prosqualodon* possessed retia (as do all modern cetaceans) is strongly suggested by certain similarities which can be demonstrated to exist between their endocasts and those of modern Mysticetes.

This being so, it is possible that certain features of the Archeocete endocast—as suggested by Marples (1949)—may be due to the presence of retia. It is clear from Dart's (1923) and Edinger's (1955) writings that no weight was given to this consideration, and that they regarded the endocast as a replica of the brain. As Edinger (1955, p. 39) states 'a palaeoneurologist calls cerebral hemisphere the endocast of the osseous chamber which once lodged the hemisphere', and on this basis both authors regarded the posterior part of the cast as representing an enormous cerebellum. Elliot Smith (1903) and Stromer (1908) had previously reached a similar conclusion. Dart (1923), in addition, emphasized an enormous trigeminal hypertrophy in the Archeocetes and to a lesser extent in *Prosqualodon*. As pointed out in the introduction, this conception of the form of the Archeocete brain is not an easy one to accept on neurological grounds, and it is desirable to examine critically the evidence on which it is based.

The whole foundation of Dart's theory of the trigeminal specialization of the Archeocetes rests upon his interpretation of the size and situation of the trigeminal ganglion. This he takes to be the relatively large mass lying alongside the cerebral hemisphere, and which, he says, it almost rivals in size. He does not advance any convincing reasons in support of this interpretation, but accepts it without further argument as a fact, and elevates it to the status of a first premise to which he relates many other of the unusual features of the cast. For example, the marked breadth of the ventral aspect of the cast of *Zeuglodon sensitivus* in the general region of the medulla oblongata is alleged to be due to expansion of the 'tuberculum quinti', the

upper portion of which is stated to form a bulge on the cast postero-medial to the 'Gasserian ganglion'. The precise situation of this bulge is doubtful since it is not labelled in any of the figures. A study of the actual cast shows two bulges in this general situation but there is nothing about either of them as regards situation and appearance which can even remotely justify regarding it as being produced by the 'tuberculum quinti' in preference to anything else.

It would not be difficult to suggest alternative explanations to account for the appearance of Dart's 'Gasserian ganglion'; for instance, it may well be due to the presence of retial tissue associated with the branches of the trigeminal nerve such as is found in modern cetaceans. Both interpretations are of necessity speculative, but whereas the one involves the acceptance (on highly questionable evidence) of a degree of hypertrophy of the trigeminal apparatus which has been seen in no other mammal, living or extinct, the other has the support of similar appearances in the endocasts of modern representatives of the same order, which undoubtedly result from the presence of retial tissue.

Perhaps the most extraordinary feature of the Archeocete endocranial cast is the massive expansion laterally and upwards of the posterior end. On the basis of its position and on theoretical grounds Elliot Smith (1903), Stromer (1908), Dart (1923) and Edinger (1955) regard almost the whole of this mass as representing the actual size of the cerebellum. Dart, in referring to the natural cast of *Z. sensitivus*, says that it 'reproduces very faithfully the convolitional pattern of the cerebellum', and proceeds to identify a 'lobus medius', a 'paraflocculus' and a 'lobulus simplex'. Examination of the actual cast reveals these elevations, but cannot by itself justify their identification. The only justification for the label 'paraflocculus' (in which is apparently included the flocculus) is its postero-lateral position in the cast, and the theoretical consideration that in all marine mammals this part of the cerebellum, which was thought to be concerned with equilibration, might be expected to be enlarged. This postulated association between 'equilibration' and the paraflocculus has not been borne out by subsequent anatomical and experimental studies (see Brodal, 1954). Now we have seen in modern cetaceans, Odontocete and Mysticete alike, that no information of any value concerning the morphological subdivisions of the cerebellum can be obtained from the endocast, and indeed, this applies to mammals in general as a glance at a number of casts will show. There is no reason therefore for the identification which has been made, apart from the theoretical expectation that an aquatic mammal would have a large cerebellum with particularly well-developed paraflocculi. If the theoretical expectation had been the opposite of this, the cast could have been interpreted in at least an equally plausible manner.

That theoretical expectations have been the main influence in making interpretations is even more evident in the case of *Prosqualodon*. Here again a huge cerebellum is recognized, although in this instance with even less justification than in the Archeocete cast, since, to judge from his figure (1923, text-fig. 18) there is little to indicate even an approximate line of subdivision between cerebrum and cerebellum. The cast from a foetal fin-whale shows that a brain of an entirely different form to that postulated for *Prosqualodon* can be associated with a very similar endocranial cast. In the fin-whale the differences between cast and brain can be attributed to the

presence of retia (much less developed than in the adult) and, since they are such as to make the cast closely resemble that of *Prosqualodon*, it is quite possible that retia were present in this animal as well and to a large extent determined the form of the endocranial cast.

The neurological considerations which have led Dart and others (e.g. Edinger, 1955) to justify their interpretation of the features of the casts merit some discussion. As previously indicated, both these authors considered that the Archeocetes must have possessed a large cerebellum because of the needs of 'equilibration', a consideration partly based upon the fact that modern marine mammals have large cerebella. Enlargement of the cerebellum, however, can result from a number of causes other than the need for equilibration. Structures directly concerned with equilibration in Cetacea such as the vestibular apparatus and the vestibular division of the 8th nerve are small. The flocculo-nodular lobe of the cerebellum, the part which receives direct vestibular fibres, is manifestly atrophic (Jansen, 1953), and the great size of the modern cetacean cerebellum is due mainly to enlargement of the paraflocculus.

It is now generally agreed that the large paraflocculus of modern cetaceans is associated with connexions from the upper levels of the brain stem or cerebral hemisphere (Brodal & Jansen, 1954). Dow (1942) found action potentials in the paraflocculus following stimulation of the cerebral cortex, and Wilson (1933) estimates that 60% of the ponto-cerebellar fibres pass to the paraflocculus in the blue whale. Bearing these facts in mind, it seems that the enlargement of the paraflocculus of cetaceans should be associated with the enlargement of the cerebral hemispheres (certainly a characteristic of all modern representatives) rather than with any paramount need for equilibration. According to Dart and others, the cerebral hemisphere and pons are small in the Archaeocetes. If one accepts these small cerebral hemispheres and pons it is difficult to associate them with an enormously hypertrophied cerebellum.

Dart also postulates that the bulk of the trigeminal fibres ended in the cerebellum, on the basis of his theory that in the zeuglodonts 'equilibration' was subserved by this nerve, and it is in this manner that he associated the alleged enormous development of trigeminus and cerebellum. The suggestion that the trigeminus could take over equilibratory function is purely speculative and direct trigemino-cerebellar fibres are known to be very few in mammals (Brodal, 1954). If they were preponderant in the zeuglodonts then this is just another instance of an almost incredible uniqueness of the neurological make-up of these animals.

A considerable part of current palaeontological opinion concerning the manner of life, affinities and relationships of the Archeocetes is at least partly based on the interpretation of endocranial casts. As a result of the present investigation and discussion one is led to conclude that these casts are unlikely to have reproduced with any degree of accuracy the form of the brain. One could probably go so far as to state that conclusions based upon them are probably worthless, and that far from contributing anything of value towards solving the problems of the morphology of the cetacean brain they are more likely to have clouded the issue. One cannot say that the Archeocete brain was necessarily similar to that of other mammals living at the same time; on general grounds it is likely that it already possessed specializations which may or may not have resembled those of the modern cetacean brain;



one can say, however, that the view that it differed so radically in particular ways from all other mammalian brains is based on highly questionable evidence and improbable speculation. This view, reached from a different approach agrees with that advanced by Marples (1949), whose paper directly led to this investigation.

If, as has been suggested here, the Archeocete casts are to be rejected as misleading, it is pertinent to inquire what one's attitude should be towards the more recent fossil cetacean casts which have been described. As regards *Prosqualodon* (Miocene) it would seem evident that Dart's (1923) interpretation is unlikely to be correct. Marples (1949) compared vascular impressions which he states can be identified on the cast with those evident on casts from recent Odontocetes, and concluded that the cerebrum was larger, and the cerebellum smaller and more ventrally placed. This much more likely interpretation, must, however, in the absence of further evidence, remain no more than a suggestion incapable of being fully proven. Indeed, it is probable that the cast of *Prosqualodon* is no more likely to yield information of value than are the Archeocete casts. As regards the more recent Miocene casts such as that of *Cyrtodelphis sulcatus* (Dal Piaz, 1905) the position is somewhat different. The form of this cast bears a reasonably good resemblance to that of a mammalian brain, and although many of Dal Piaz's more detailed identifications are open to question, his general conclusions by no means stretch credulity unduly. In fact, it is likely that what has been said (p. 541) concerning the casts of the modern smaller Odontocetes should apply equally in this instance, i.e. that the cast probably gives a reasonably true impression of the general proportions of the major parts of the brain with some distortion of detail. It may be noted in passing that the 'cerebellar' portion of this cast bears a close resemblance in shape to the corresponding region of the cast of the porpoise, a fact which strongly suggests the presence of retial tissue in this situation in the former.

It is interesting to note that in the case of both recent and fossil cetaceans, endocranial casts of two types are encountered. On the one hand are those of the Archeocetes, *Prosqualodon*, modern Mysticetes, and the sperm whale, which give a very poor impression of the form of the brain, and on the other the casts of the Miocene Odontocetes and the smaller modern Odontocetes from which a less distorted impression may be gained. It will be noted that this grouping cuts across both time and taxonomy, and that casts of both types are encountered among fossil as well as recent cetaceans. Since the differences in the case of the latter can be attributed to varying degrees of development of the intracranial vascular structures, it is conceivable that a similar state of affairs might have obtained in the fossils, and thus account for the more bizarre appearance of some of the casts without implying an extraordinary development of certain parts of the brain. Even among the Archeocetes as a group, marked differences in appearance occur. These have been interpreted by Dart (1923) as indicating progressive trigeminal specialization and degeneration. Might they not be equally well accounted for by attributing them to differences in the degree of development of retial tissue in different members of the group?

## SUMMARY

1. Endocranial casts of living cetaceans can at best give only a very general impression of the form and proportions of the brain. Where retia mirabilia are particularly well developed, e.g. as in Mysticeti, such casts are positively misleading if regarded as reasonable replicas of the brain.

2. Certain features common to the endocranial casts of the Archeocetes (as well as *Prosqualodon*) and those of recent Mysticetes, strongly suggest the presence of retia in the former group. It is suggested therefore that these casts are unlikely to be of any assistance towards elucidating the true form of the Archeocete and Squalodont brains, and that previous views concerning the size of the cerebellum and trigeminal apparatus in these forms are highly speculative and based upon inadequate evidence. Certain neurological considerations would appear to reinforce this view.

I should like to express thanks to Dr E. Downing, Senior Chemist, Messrs Chr. Salvesen and Co., Leith, for providing foetal and adult whale material used in this investigation. Thanks are also due to Drs A. T. Hopwood, F. C. Frazer and P. E. Purvess of the British Museum (Natural History) for access to material, and finally to Prof. F. Goldby for advice and encouragement throughout. The text-figures were drawn by Miss Jill Payne, and the photographs for the plate were provided by the Photographic Department, St Mary's Hospital Medical School.

## REFERENCES

- BRODAL, A. (1954). Afferent cerebellar connections. In *Aspects of Cerebellar Anatomy*, by Jansen, J. and Brodal, A. Oslo: Johan Grundt Tanum Forlag.
- BRODAL, A. & JANSEN, J. (1954). Structural organisation of the cerebellum. In *Aspects of Cerebellar Anatomy*, by Jansen, J. and Brodal, A. Oslo: Johan Grundt Tanum Forlag.
- DAL PIAZ, G. (1905). Sugli Avanzi di *Cyrtodelphis sulcatus* dell' arenaria di Belluno. Pt. 2. *Palaeontogr. ital.* **11**, 253-280.
- DART, R. A. (1923). The brain of the *Zeuglodontidae*. *Proc. zool. Soc. Lond.* pp. 615-654.
- DOW, R. S. (1942). Cerebellar action potentials in response to stimulation of the cerebral cortex in monkeys and cats. *J. Neurophysiol.* **5**, 121-136.
- EDINGER, T. (1955). Hearing and smell in cetacean history. *Msehr. Psychiat. Neurol.* **129**, 37-58.
- ELLIOT SMITH, G. E. (1903). The brain of the *Archeoceti*. *Proc. Roy. Soc.* **71**, 322-331.
- FLYNN, T. T. (1947). Description of *Prosqualodon davidi* Flynn, a fossil cetacean from Tasmania. With a note on the microscopic tooth structure by T. Thornton Carter. *Trans. zool. Soc. Lond.* **26**, 153-197.
- GERVAIS, P. (1871). Remarques sur l'Anatomie des Cétacés de la division des Balénidés. *Arch. Mus. Hist. nat. Paris*, **7**, 65-146.
- JANSEN, J. (1953). Studies on the cetacean brain. The gross anatomy of the rhombencephalon of the fin-whale (*Balaenoptera physalus*, L.). *Hvalråd. Skr.* **37**, 1-35.
- KELLOGG, R. (1928). The history of whales—their adaptation to life in the water. *Quart. Rev. Biol.* **3**, 29-76, 174-208.
- KELLOGG, R. (1936). A review of the Archaeoceti. *Publ. Carneg. Instn.* **482**, 1-366.
- MARPLES, B. J. (1949). Two endocranial casts of cetaceans from the Oligocene of New Zealand. *Amer. J. Sci.* **247**, 463-471.
- STROMER, E. (1908). Die Archaeoceti des ägyptischen Eozäns. *Beitr. Paläont. Geol. Öst.-Ung.* **21**, 106-177.
- WALMSLEY, R. (1938). Some observations on the vascular system of a female fetal finback. *Publ. Carneg. Instn.* **496**, 107-178.
- WILSON, R. B. (1933). The anatomy of the brain of the whale (*Balaenoptera sulfurea*). *J. comp. Neurol.* **58**, 419-480.

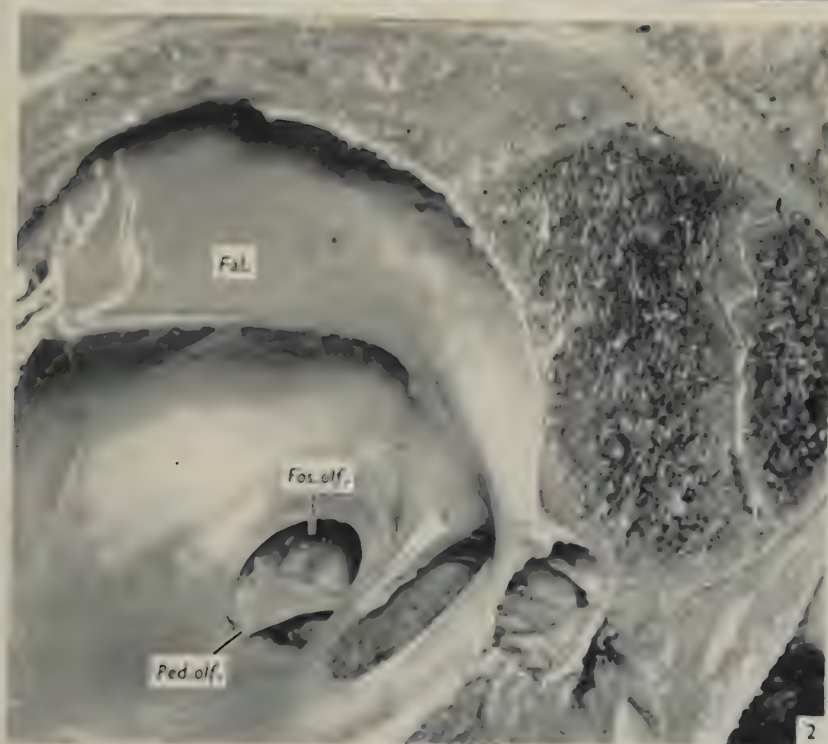
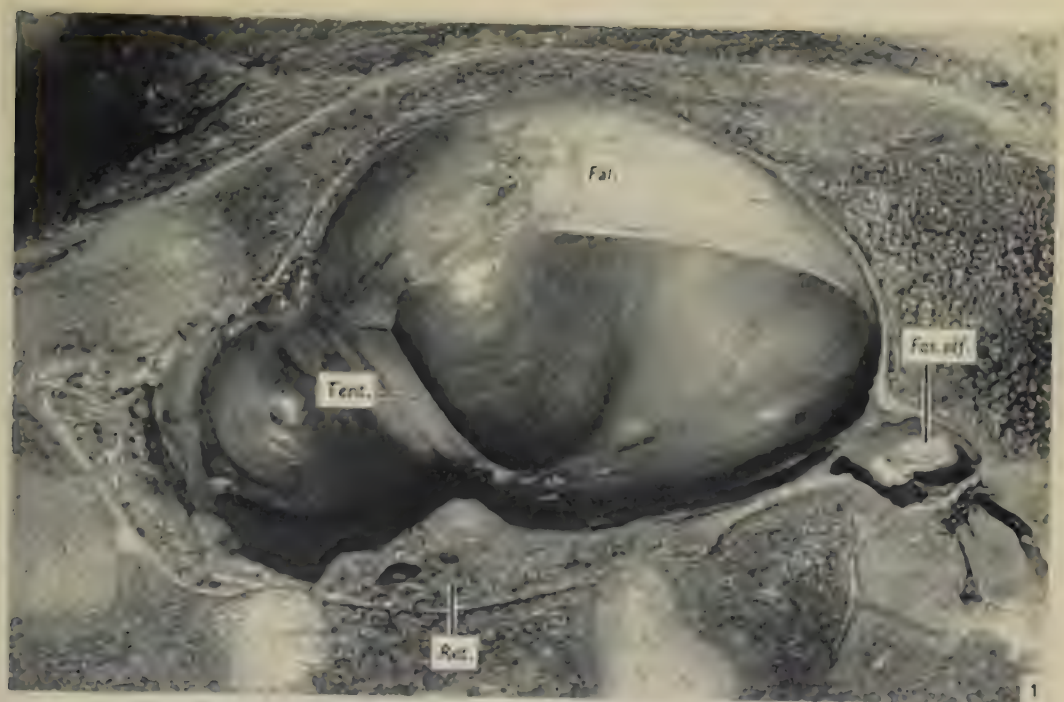
## EXPLANATION OF PLATE

*Abbreviations:* *Fal.*, falx cerebri; *Fos.olf.*, olfactory fossa; *Ped.olf.*, olfactory peduncle; *Ret.*, rete mirabile; *Tent.*, tentorium cerebelli.

Fig. 1. Parasagittal section of skull of foetal fin-whale (*Balaenoptera physalus*) to show relation of olfactory fossa to main endodural space.  $\times$ , about 0.6.

Fig. 2. Anterior portion of endodural space of the same specimen viewed from above and behind. There is considerable disproportion between the size of the olfactory peduncle (enveloped in membranes) and that of the olfactory fossa. About natural size.







## THE BLOOD CAPILLARY SYSTEM OF THE ODONTOBLAST LAYER OF THE DENTAL PULP

By W. WARWICK JAMES, O.B.E., F.R.C.S.

*Department of Anatomy, Middlesex Hospital Medical School*

The one function of the tooth pulp is to ensure the production of dentine by the odontoblasts, specially differentiated mesenchyme cells situated upon the surface beneath the enamel organ. Now while it has long been known that the pulp is richly vascular, information on the distribution of the capillaries upon which the nutrition of the odontoblasts depends is scanty indeed.

Most observations on the capillaries to the odontoblasts have been made on human material. Meyer-Churchill (1935), in figures 108 and 110, shows both the main central vessels of the pulp and also capillaries proceeding from them to end as terminal loops between the odontoblasts. Orban (1944), recognized the increasing vascularity of the pulp during tooth development, and in the formed tooth observed capillary loops close to the odontoblasts and even extending into that layer. Kramer (1951) described a capillary plexus beneath the odontoblast layer on material which had been injected by sucking indian ink into the pulp vessels.

Thus capillaries in direct relation with the odontoblasts have certainly been observed in human teeth, and similar appearances are suggested in rat material figured by Kreshover, Clough & Bear (1953).

In the present investigation mammalian, reptilian, amphibian and fish teeth have all been found to demonstrate a very close relationship between capillaries and odontoblasts. The importance of this observation was realized when comparison was made with the work on the development and repair of bone by Ham (1952). By measuring the distance of the osteoblasts and osteocytes from the blood vessels he found that the cells and capillaries are approximately  $\frac{1}{10}$  mm. apart, and also that the canalicular mechanism cannot operate effectively over a greater distance. The nutritional conditions for bones cells are fully discussed by Ham in his *Histology* (1953). In the present study measurements of the distance between the odontoblasts and the capillaries were not made; however, when in the wide range of animals examined it was found that all showed the very close association between odontoblasts and capillaries the principle established by Ham for bone seemed to be justified for dentine as well. Differences which have been observed appear to depend upon such variable factors as the size of the odontoblasts and of the capillaries (which are large in forms possessing nucleated erythrocytes), and upon variation in the tissue of the different animal classes.

Through the kindness of Dr I. C. Michaelson, indian ink injected specimens of cat, kitten, rat and hamster prepared by him for his study of the retina (1949) were made available. In some of these specimens the finest capillaries amongst the odontoblasts had been filled.

The hamster incisor shown in Pl. 1, fig. 1, is quite striking. Very fine capillaries can be seen, both amongst the odontoblasts and also on their outer aspect running



parallel to the dentinal surface; immediately deep to the odontoblasts larger vessels are also shown running parallel to the surface, these being in obvious continuity with central vessels lying, as is usual, in the long axis of the pulp. A somewhat similar appearance is shown in the injected pulp of a kitten (Pl. 1, fig. 2).

In sections of material not previously injected (Pl. 1, figs. 3-7) it is still possible to identify capillaries, amongst the odontoblasts or in contact with them, by the presence of red blood corpuscles surrounded by endothelium. When they are empty and collapsed identification of capillaries is difficult, depending then solely on the presence of endothelial cells.

In the cod, Pl. 1, fig. 3, and the iguana, Pl. 1, fig. 4, capillaries in close relation to the odontoblasts are easily seen, the nucleated erythrocytes and the endothelial cells standing out clearly. In the puff adder, Pl. 1, fig. 5, red cells are present in some capillaries but not all; several empty vessels show up very well but many finer capillaries empty of erythrocytes tend to escape notice. In the foetal lion (Pl. 1, fig. 6) the pulp capillaries in relation to the odontoblasts do not all contain red cells, but even so it is possible to trace very fine capillaries among the odontoblasts. In the cat (Pl. 1, fig. 7) capillaries cut in section within the odontoblast layer are easily seen.

It is surprising that in injected specimens the pulp capillaries are not more readily filled, for one gets the impression from examining sections of uninjected material that they are quite plentiful. Possibly the general pressure in the larger pulp vessels is transmitted laterally to the walls of the pulp capillaries and so reduces their calibre. It may be that the capillaries amongst the odontoblasts are more firmly supported and in consequence not so easily compressed as those of the pulp; the injected hamster and kitten material lends weight to this argument. Certainly the smaller vessels of the pulp are less evident than might be expected. For instance, the number of vessels seen in foetal lion section suggests a very free supply, and most probably many more are present than those that can certainly be identified. Lateral compression may account for capillaries among the odontoblasts not being reached by the injection in Kramer's (1951) experiment, although a negative pressure might have been thought to be more favourable than a positive one. His explanation of the presence of red cells is possible.

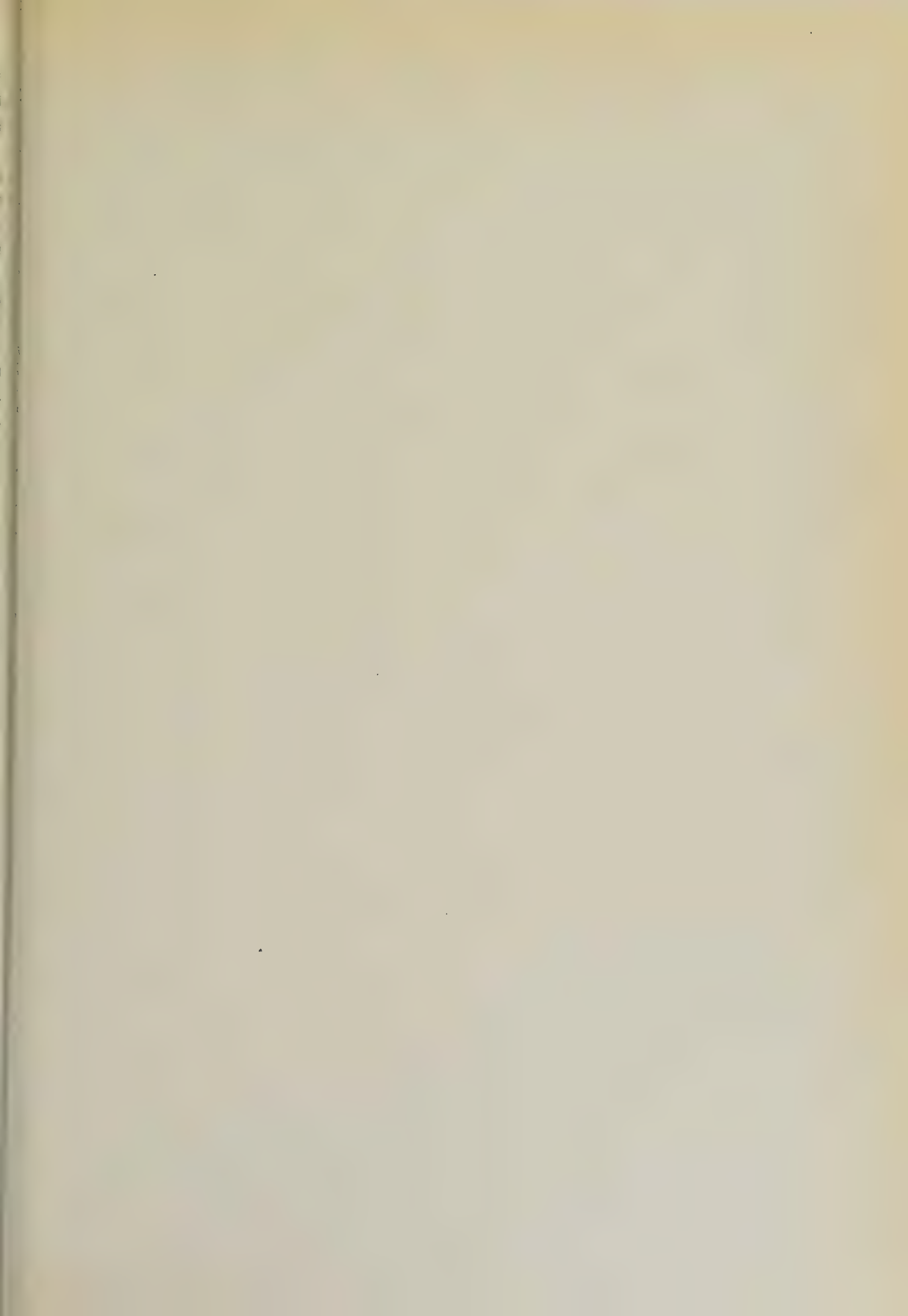
A difference in vascularity might be expected between the teeth of polyphyodonts with open bases and those of diphyodonts with contracted roots. However, during development the base of the diphyodont tooth is open and, in the case of persistently growing teeth such as incisors, stays open.

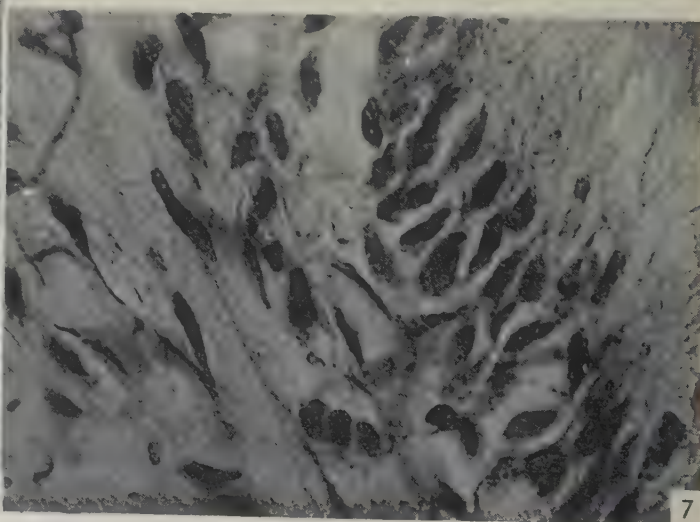
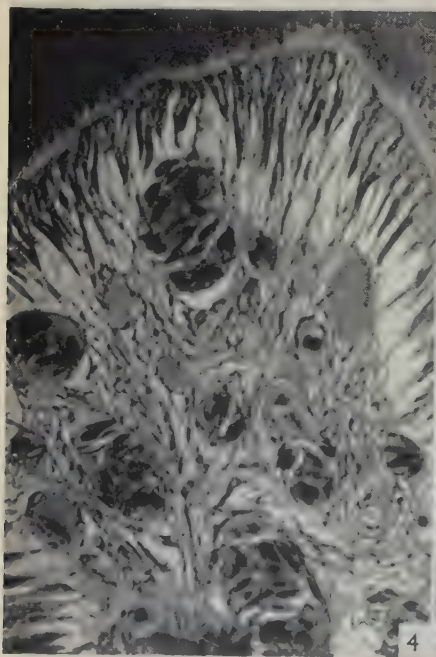
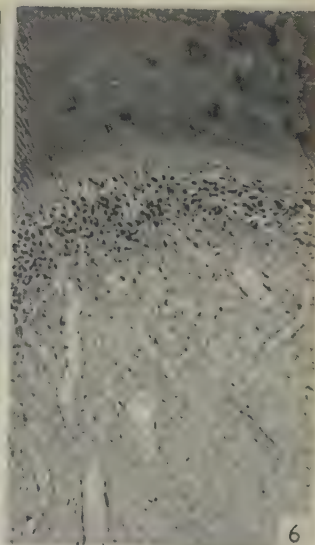
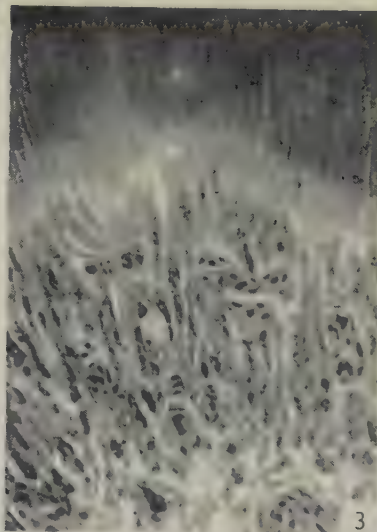
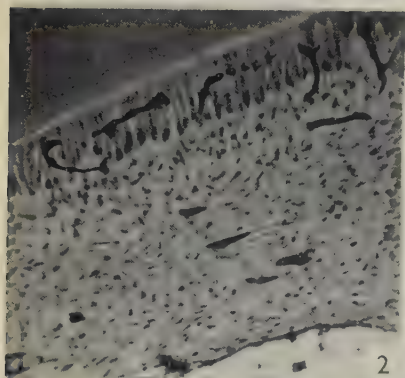
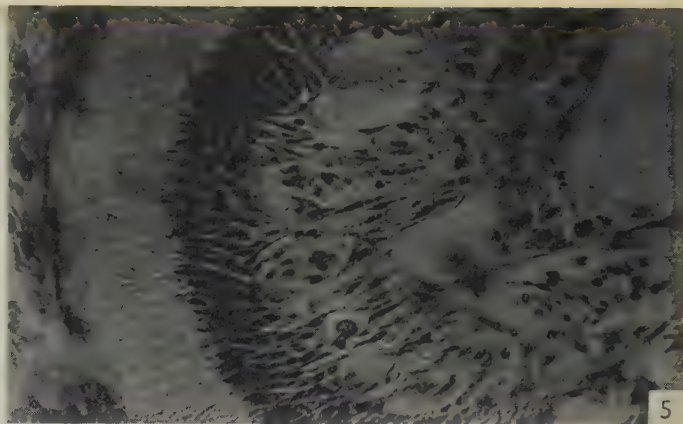
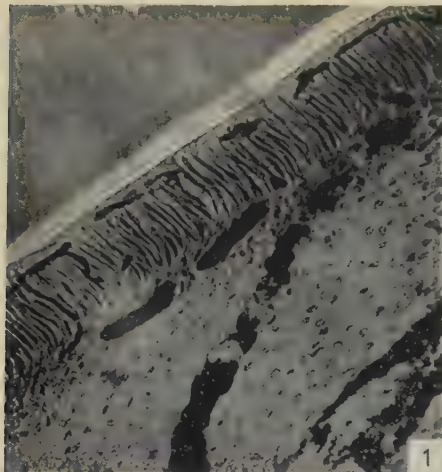
#### SUMMARY

The close association of the capillaries of the pulp to the odontoblasts has been demonstrated in sections of the teeth of mammals, reptiles, amphibia and fishes.

Attention has been directed to the nutritional importance of this arrangement.

The relationship has been compared with that of the blood vessels to osteogenetic cells in bone.





WARWICK JAMES—THE BLOOD CAPILLARY SYSTEM OF THE ODONTOBLAST LAYER OF THE DENTAL PULP



# REFERENCES

- CHURCHILL, H. R. (1935). *Meyer's Normal Histology and Histogenesis of the Human Teeth and Associated Parts*. Philadelphia: J. B. Lippincott and Co.
- HAM, A. W. (1952). Some histophysiological problems peculiar to calcified tissues. *J. Bone Jt. Surg.* **34A**, 701-728.
- HAM, A. W. (1953). *Histology*. Philadelphia: J. B. Lippincott and Co.
- KRAMER, I. R. H. (1951). A technique for the injection of blood vessels in the dental pulp using extracted teeth. *Anat. Rec.* **111**, 91-100.
- KRESHOVER, S. J., CLOUGH, O. W. & BEAR, D. M. (1953). Prenatal influences on tooth development. I. Alloxan diabetes in rats. *J. dent. Res.* **32**, 246-261.
- MEYER-CHURCHILL, see CHURCHILL.
- MICHAELSON, I. C. (1949). Vascular morphogenesis of the retina of the cat. *J. Anat., Lond.*, **83**, 64.
- ORBAN, B. (1944). *Oral Histology and Embryology*. London: Henry Kimpton.

## EXPLANATION OF PLATE

- Fig. 1. Hamster (Weigert's haematoxylin and van Gieson) ( $\times 385$ ). Vessels of a persistently growing incisor injected with indian ink through the carotid artery. The finest capillaries have been reached, and those parallel to the surface of the forming dentine are well shown. Larger vessels in communication with the central vessels of the pulp are seen along the bases of the odontoblasts. It is remarkable that the finer capillaries of the pulp are not injected.
- Fig. 2. Kitten (haematoxylin and eosin) ( $\times 165$ ). Incisor vessels injected with indian ink through the common carotid artery. Amongst the odontoblasts fine capillaries parallel to the surface of the forming dentine are shown. Surprisingly few capillaries are injected in the pulp.
- Fig. 3. Cod (haematoxylin and eosin) ( $\times 385$ ). Numerous large capillaries of the pulp are shown in close relation to the odontoblasts where they are most plentiful; they have the appearance of forming loops. The large nucleated erythrocytes are distinct.
- Fig. 4. Iguana (Weigert's haematoxylin and van Gieson) ( $\times 385$ ). Large capillaries, with nucleated erythrocytes, are shown closely related to the odontoblasts. There is a very full vascular supply to the pulp.
- Fig. 5. Puff adder (haematoxylin and eosin) ( $\times 385$ ). Numerous capillaries of the pulp are shown in close contact with the odontoblasts; some contain erythrocytes, others are empty showing only endothelial cells. The very fine vessels running across the odontoblasts seen in the hamster do not appear to be present.
- Fig. 6. Foetal lion (haematoxylin and eosin) ( $\times 90$ ). Pulp capillaries are shown in relation to the odontoblasts and although for the most part empty their endothelial cells are distinct. Very fine capillaries can be traced among the odontoblasts. The large number of capillaries in the body of the pulp is in contrast with the relatively few which show in injected mammalian material.
- Fig. 7. Cat (Weigert's haematoxylin and van Gieson) ( $\times 1000$ ). Capillaries are shown both in the pulp and amongst the odontoblasts.

## THE INSERTION OF THE BICEPS FEMORIS

BY R. S. SNEATH

*Department of Anatomy, University of Sheffield*

The tendon of insertion of the biceps femoris is usually described as split into two parts, *anterior* and *posterior*, by the lateral ligament of the knee joint. Some authors, however, have described the tendon as merely grooved on its deep surface by the ligament (Fick, 1904). Last (1948) maintained that the tendon is not split; he also described an extension of the tendon passing to the fibula, lying deep to the lateral ligament, between it and the true capsule of the knee. The present work indicates that the description of the insertion of the tendon should be revised.

Dissections were made of forty-four knee joints of dissecting room cadavers, ranging in age from 13 to 95 years. A freshly amputated knee of an adult and the knees of a full-term foetus were also dissected. In addition, the flexed knee of an embryo of 118 mm. c.r. length was serially sectioned, the sections passing coronally through the thigh and transversely through the leg.

### OBSERVATIONS

The long head of the biceps has a fusiform muscle belly, which in the adult blends with its tendon 3-4 in. above the knee joint. This tendon is flattened from side to side, and into its deep surface and anterior border are inserted the muscle fibres of the short head. The tendon (Fig. 1, *B.T.*) passes downwards and forwards to be inserted into the head of the fibula, the lateral ligament of the knee and the lateral condyle of the tibia. The tendon blends anteriorly with the ilio-tibial tract and gives off expansions to the crural fascia covering the anterior, lateral and posterior compartments of the leg. The part of the tendon that is inserted into the fibula (Fig. 1, *F.*) is derived principally from the long head and wraps round the lateral, posterior and medial surfaces of the lateral ligament of the knee (Fig. 1, *L.*). The tendon is not actually split by the ligament, though it is sometimes thinned where this ligament comes nearest to the surface.

The remaining part of the tendon, which is derived mainly from the short head, forms three laminae, superficial, intermediate and deep (Fig. 1, *S.L.*, *I.L.*, *D.L.*). The superficial lamina passes superficial to the lateral ligament of the knee and is inserted into the lateral condyle of the tibia (Fig. 2, *S.L.I.*). The intermediate lamina blends with the posterior border of the distal third of the lateral ligament. The deep lamina passes deep to the lateral ligament, separates it from the popliteus tendon and the inferior lateral genicular vessels, and then is inserted into the lateral condyle of the tibia (Fig. 2, *D.L.I.*) immediately behind, and blending with, the insertion of the superficial lamina. The superficial and deep laminae, together with the fibres of the anterior ligament of the superior tibio-fibular joint (Figs. 1, 2, *T.F.*), form a conjoint band inserted into a rough area on the lateral condyle of the tibia, immediately posterior to the attachment of the ilio-tibial tract (Figs. 1, 2, *I.T.*). Covering

the medial, anterior and lateral aspects of the lower third of the lateral ligament is a constant synovial bursa (Fig. 1, *B.B.*) which separates the ligament from the superficial and deep laminae of the tendon.

There is considerable variation in the proportion of the fibres of the biceps tendon which constitute the individual laminae. Approximately half of the tendon fibres are inserted into the fibula, rather less than one half into the tibia and the remainder into the crural fascia.

In the full-term foetus the arrangement is similar to that in the adult, including the presence of a bursa around the lateral ligament. In the 118 mm. embryo the tendon also has the same arrangement of its three laminae, but the bursa has developed only on the superficial surface of the ligament.

#### ABBREVIATIONS USED IN FIGURES

<i>B.B.</i>	Biceps bursa related to lateral ligament of knee.
<i>B.T.</i>	Tendon of biceps femoris.
<i>D.L.</i>	Deep lamina of the biceps tendon.
<i>D.L.I.</i>	Insertion of the deep lamina.
<i>F.</i>	Fibular part of biceps tendon.
<i>F.I.</i>	Insertion of the fibular portion of the tendon.
<i>I.L.</i>	Intermediate lamina of the biceps tendon.
<i>I.T.</i>	Insertion of the ilio-tibial tract and lateral patellar retinaculum (derived from vastus lateralis).
<i>L.</i>	Lateral ligament of the knee.
<i>L.F.</i>	Fibular attachment of the lateral ligament of the knee.
<i>S.L.</i>	Superficial lamina of the biceps tendon.
<i>S.L.I.</i>	Insertion of the superficial lamina of the tendon.
<i>T.F.</i>	Anterior ligament of superior tibio-fibular joint.

#### DISCUSSION

During movement of a tendon in relation to its bony attachment, angulation occurs in each fibre between its intra-tendinous mobile portion and its intra-osseous non-mobile portion. If the tendon fibres were tensed throughout their range of movement, an abrupt point of transition from the mobile to the non-mobile portions would cause excessive wear and fraying at this point. This weakness is overcome in a number of ways which were described by Mollier (1937) in his study of tendons generally. The commonest method is a fanning of the tendon attachment, so that, in any position of the joint, there will be a number of fibres in which the mobile and non-mobile portions of each fibre are in direct line. These fibres, due to their interweaving within the tendon as described by Mollier, are acted upon by the whole of the muscle. The remainder of the fibres of the tendon are slack, and wear at the point of transition is thus reduced to a minimum.

The insertion of the biceps is an example of a fan, with an upper horizontal border composed of the fibres that are inserted into the tibia, and a lower vertical border, composed of the fibres that are inserted into the fibula. In most mammals and primates the short head of the biceps, with which the tenuissimus is homologous (Klaatsch, 1902), is inserted distal to the long head, but in man these insertions, besides becoming concentrated on the head of the fibula, have become reversed.



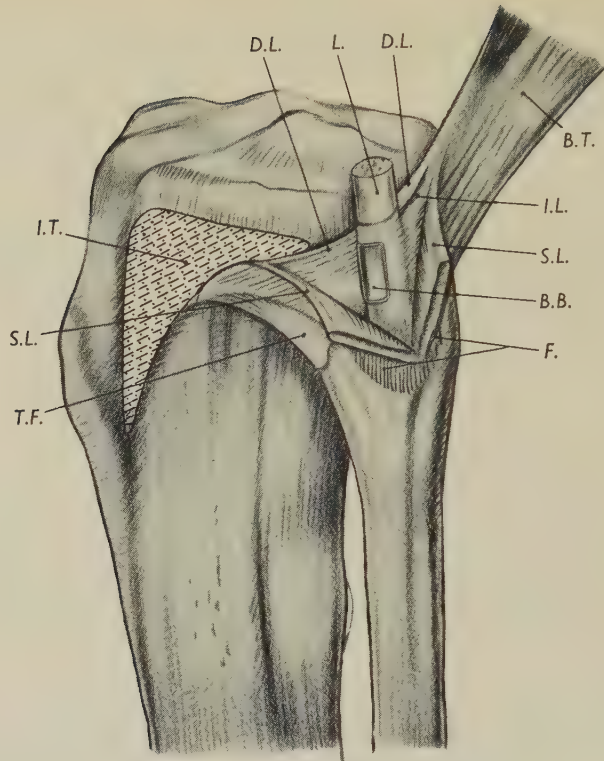


Fig. 1. Lateral view of tibia and fibula to show arrangement of biceps tendon.

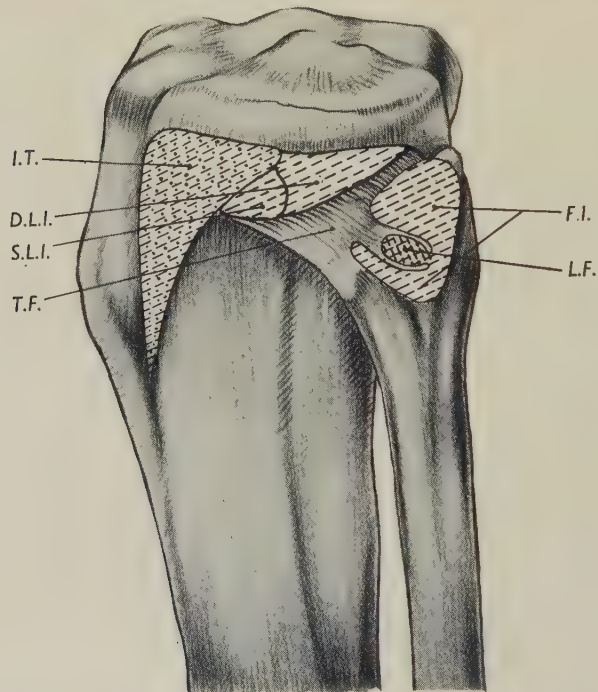


Fig. 2. Lateral view of tibia and fibula to show bony attachments of biceps tendon.

It is of interest to note that the long head of the biceps can produce full extension of the hip when the knee is extended, but increasing degrees of flexion of the knee proportionately decrease this range of extension, because this head of the muscle is one of short action (Haines, 1934). Similarly, extension of the hip decreases the possible flexor action of the long head of the biceps on the knee. So, with a fully extended hip, complete flexion of the knee by the biceps can only be produced by its short head (Paterson, 1917). It is the upper horizontal group of fibres in the fan that are tense in full flexion, and it is these that receive the major part of the attachment of the short head of the biceps.

The attachment of the biceps tendon to the lateral ligament of the knee may be of mechanical significance. Fick (1911) states that the lateral ligament is taut in extension of the knee, but that, after the first few degrees of flexion, it becomes slack and remains so up to full flexion. The present work suggests that, in active flexion of the knee, the intermediate lamina of the tendon would keep the ligament taut by bowing it. In the dissected specimens it was found that when the knee was placed in a flexed position, the lateral ligament was slack, but that when the tendon was pulled, the ligament became bowed and taut.

#### SUMMARY

1. The insertion of the biceps femoris tendon in the human has been examined in forty-eight knees.

2. Approximately half of the tendon is inserted, without splitting, into the fibula, only a few of the fibres being inserted into the crural fascia. The remainder of the tendon is divided into three laminae, of which the intermediate is inserted into the lateral ligament of the knee, while the other two pass respectively superficial and deep to the ligament to be inserted into the lateral condyle of the tibia.

3. The significance of these findings is discussed, particularly with relation to Mollier's principle of fanned tendon insertions.

I am indebted to Prof. Francis Davies for his interest and advice.

#### REFERENCES

- FICK, R. (1904). Anatomie und Mechanik der Gelenke, Abt. I, T. 1. In *Handbuch der Anatomie des Menschen*, ed. K. v. Bardeleben, Bd. II, 368-369. Jena: Gustav Fischer.
- FICK, R. (1911). Anatomie und Mechanik der Gelenke, Abt. I, T. 3. In *Handbuch der Anatomie des Menschen*, ed. K. v. Bardeleben, Bd. II, 542-543. Jena: Gustav Fischer.
- HAINES, R. W. (1934). On muscles of full and of short action. *J. Anat., Lond.*, **69**, 20-24.
- KLAATSCH, H. (1902). Der kurze Kopf des Musculus biceps femoris und der Tenuissimus. *Morph. Jb.* **29**, 217-281.
- LAST, R. J. (1948). Some anatomical details of the knee joint. *J. Bone Jt. Surg.* **30B**, 683-688.
- MOLLIER, G. (1937). Beziehungen zwischen Form und Funktion der Sehnen in Muskel-Sehnen-Knochen-System. *Morph. Jb.* **79**, 161-199.
- PATERSON, A. M. (1917). The action of the biceps flexor cruris. *J. Anat., Lond.*, **61**, 362-363.

## REVIEWS

*An Introduction to Cell and Tissue Culture.* By the Staff of the Tissue Culture Course, Cooperstown, New York. 1949-53. Ed. W. F. Scherer. (Pp. 123; \$4.00.) Burgess Publ. Co.

During the last decade the fresh impetus which the discipline of tissue culture has experienced, particularly in the United States, may be described as a renaissance. New techniques of growing relatively large masses of tissue have led to their use in such diverse fields as the study of cell metabolism and the large-scale investigation of animal viruses. As a consequence of these developments, there has been a demand from research workers in various branches of biology for practical instruction in modern methods of tissue culture. In America this need has been met by the Tissue Culture Association, who since 1949 have organized a series of summer schools for this purpose. The present volume is a practical notebook, based on these courses, in which seven experts in different branches of tissue-culture technique describe the various methods which they have developed. To those who wish to apply such procedures to their own biological problems, and who may not be able to attend the Cooperstown Course, this book will prove invaluable.

As with most handbooks on methods of tissue culture, however, it is necessary to state that for many purposes in cytology and experimental embryology, older and much simpler methods are still adequate. On p. 19 of this volume, the classical 'hanging-drop' culture is compared by Dr J. H. Hanks to a senescent horse, though he admits that 'this formerly fancy pacer can still show some perfectly remarkable exhibitions in short runs'.

It is probable that elaboration of technical detail deters not a few biologists from attempting the cultivation of tissues where quite simple procedures could well suffice. At one time, under the late Dr Carrell, sterile precautions were carried to a forbidding extreme; the directions for cleaning glassware in the present volume exhibit a similar tendency towards technical elaboration for its own sake. It must be admitted that methods of this kind are only appropriate to a research unit endowed with extensive technical assistance.

One minor trend in the intensive development of special departments of study is the appearance of esoteric nomenclature. This again may serve as an impediment to the aspirations of a novice. Thus in this book, among other abbreviations, we find that an isotonic solution of sodium bicarbonate is denoted by the symbol NaH. The ink thus saved does not warrant such an affront to the unity of scientific usage.

ARTHUR HUGHES

*Primates. Part 2. Haplorhini: Tarsiodea.* By W. C. OSMAN HILL. (347 pp.; 49 text-figures and 14 plates; 68s.) Edinburgh University Press.

The second volume of Dr Hill's systematic work on the comparative anatomy of the Primates consists of two main parts—the first providing a survey of the morphological characters of the Haplorhini in general, and the second an account of the anatomy of *Tarsius* and fossil tarsioids. The difficulties inherent in Pocock's classification of the Primates are made apparent in the first part by the fact that in so many features does *Tarsius* appear to align itself more closely with the lemurs than with the higher Primates, and this is made still more evident from a consideration of some of the fossil tarsioids. Indeed, as the author himself emphasizes, it is quite impossible in some cases to decide on the taxonomic position of many of the fossil specimens (most of which are extremely fragmentary), and it is even probable that some of them have no relationship at all to either of the two major taxonomic categories represented by the modern lemurs and the modern tarsier. Perhaps the only certain allocation among these fossils is *Pseudoloris* which in dental and skeletal characters is so similar to *Tarsius* that, on the usual morphological



criteria, its generic distinction is extremely doubtful, and there can surely be no justification for excluding it from the family Tarsiidae. But here Dr Hill follows Simpson who has provisionally allocated this Eocene prosimian to the Anaptomorphidae (= Microchoeridae) on the grounds that it is so distant in time and space from the modern *Tarsius*. But does not this obscure the very interesting and evident fact that *Tarsius* (or the family to which it belongs) really does represent a most remarkable survival from Eocene times? In a work of this kind, however, the assembling of all the available anatomical evidence is no doubt much more important than its interpretation in terms of taxonomy (seeing that such interpretations in the absence of a tolerably complete fossil record are bound to be evanescent), and the author has certainly done a most valuable service to the whole subject of primatology by collecting together this evidence in such an accessible form. As a work of reference it will be found quite essential for every student of the subject, if only because there exists no other single work of reference which covers the same field. The fact that it also embodies many of the author's original observations still further enhances its value.

Every reviewer likes to have his little grumbles about a book, however much it is to be commended, and we are no exception. For, by a searching scrutiny, we have managed to find here and there a few scattered statements which we would be prepared to dispute with the author, though we freely admit that they are not very numerous. But we would like to ask that in future volumes the magnifications of the illustrations should in every case be indicated (particularly those of fossil specimens); and it seems a pity that the author should have reproduced the crude and inaccurate drawing of *Tetoniuss* published by Hubrecht over half a century ago, rather than Matthew's excellent illustration—a pity, because skulls or portions of skulls of fossil prosimians are so exceedingly rare. Let it be said, however, that such criticisms as these have not disturbed our appreciation of this volume, nor our pleasant anticipation of the next volume of the series which is to deal with the platyrrhine monkeys.

W. E. LE G. CLARK

*Analysis of Development*. Edited by B. H. WILLIER, P. A. WEISS and V. HAMBURGER.  
(Pp. 735; 248 figures; 105s.) Philadelphia and London: W. B. Saunders Co.,  
1955.

This book is a collection of articles by twenty-eight American biologists whose high aim has been 'to present a modern *synthesis* of our knowledge of the principles and mechanisms of development.' The scope of the work has a breadth in conformity with this purpose. The embryology of the Invertebrata receives considerable attention; sections are devoted to each of the main organ-systems of the vertebrate embryo, and the range of experimental treatment extends as far as the immunological aspects of development. The goal of the authors has been none less than to 'transcend descriptive embryology and blend experimental data with "Beobachtung und Reflexion"'. Clearly the full achievement of such an aim would mark a notable step in the progress of Embryology.

The task of a reviewer is to attempt some estimate of how far this ambition has been achieved. The final result, as the editors admit, remains inevitably a collection of separate articles. This might not seriously detract from the value of the whole work, if something near a uniformity of standard had been maintained throughout, but it is doubtful whether such a claim could be advanced with conviction. The articles differ widely in length, in their relevance to the specific problems of development, and in their proximity in time to other reviews of similar scope. Most, though not all, of the longer chapters are authoritative expositions of the present position in their own fields. Dr Jane Oppenheimer's introductory chapter on the history of embryology is of great interest. Her account of the development of the subject during the nineteenth century merits a much extended treatment.

The shorter articles in this book, however, are necessarily confined to elementary generalities. Thus, to take one example, Dr Schour's seven pages on 'Teeth' contain nothing that cannot be found in standard text-books of Histology.

Again, the distinguished American researches in the field of Neuro-embryology have

been reviewed during the last few years on several occasions. It is thus a considerable disappointment to find that Dr Weiss's chapter on Neurogenesis follows much the same lines of treatment with which one is already familiar. Nor, indeed, is this now always wholly adequate; on the subject of the growth of the nerve fibre, the author makes no reference to work in this field published within the last few years, which does not support his views on the mechanism of extension of these processes. Moreover, Dr Weiss's schemata of the postulated ultra-structure of plasma clots and the relation of cultivated nerve fibres thereto were an important contribution to the field of neurogenesis some twenty years ago, but have long since needed the test of observational evidence.

For the English reader, it is impossible not to regard these unevennesses in the light of the cost to him of the whole work. At five guineas, this book is outside the range of the undergraduate student and of most of his teachers. It will fill a place of value on the library shelves, but in the reviewer's opinion, the usefulness of these articles would have been vastly increased had they been issued in the form of a series of monographs. Most of us would have liked to buy several numbers of such a series, and in the library, subdivision would have enabled a much fuller use of the whole.

ARTHUR HUGHES

*Atlas postmortale Angiogramme.* By PROFESSOR DR J. SCHOENMACKERS and DOZENT DR H. VIETEN. (Pp. 203; 145 illustrations; DM. 57. 1954.) Stuttgart: Georg Thieme Verlag.

This atlas consists of a series of plates showing the X-ray appearances of the blood vessels of various organs which have been injected post-mortem with radio-opaque material. The injections were made into the cadaver with the organs undisturbed, so that the relationship of the vessels to the adjacent skeletal and other tissues may be seen. Each section starts with a post-mortem angiogram designed to show the normal vascular pattern. This is followed by a number of plates to show the change in vascular pattern caused by various pathological processes. Beneath each angiogram is a short description of the features to be observed and also of the pathological diagnosis.

In the section on the lung, for instance, post-mortem angiograms are given of cases of emphysema, of tuberculosis with cavity formation, of mitral stenosis, and so forth.

In addition to displaying diseased conditions of the lung, the brain, the heart and the kidney, an attempt is made to depict changes in the vessels of the thoracic cage and the abdominal contents in the same way. Three plates are given up to showing the pattern of vessels in a normal foetus and two cases of conjoined twins. Finally, venograms of the liver and of the general circulation are given.

On the whole, the quality of the X-rays is high.

The authors show a curious lack of critical judgement, for they accept the various differences in calibre of vessels seen in different cases at their face value. Again, the irregularities in filling and the apparent irregularities in the outline of normal vessels which are such common findings in injections of perfectly normal organs are not stressed by the authors, so that the reader is left with the impression that post-mortem angiograms give a reliable picture of the circulation. This is only true in the positive sense, i.e. when a vessel is well filled; but when a vessel is only partly filled or is unfilled this can be by no means accepted as showing that there is organic obstruction. The path of the injector is beset by pitfalls, and this reviewer considers that this book makes light of them.

It is hard to see who will be much benefited by owning such a book—presumably the radiologist doing angiography in the living; but he would be well to beware of the possible artefactual appearances in post-mortem angiograms.

There is a short summary in German, English, French and Spanish at the end of each section. The bibliography is very slight, and it seems a pity that the excellent little *X-ray Atlas of the Systemic Arteries of the Body* published by H. C. Orrin in 1920 is not referred to, for it must be one of the earliest attempts to show the vessels of the human body by post-mortem angiography.

P. DANIEL

## PROCEEDINGS OF THE ANATOMICAL SOCIETY

NOVEMBER 1954

The Annual General Meeting of the Society for the Session 1954-5 was held in the Department of Anatomy, Guy's Hospital Medical School, London Bridge, S.E. 1, on Friday, 26 November 1954. The President (Prof. W. J. HAMILTON) was in the Chair.

The following are the authors' abstracts of the papers presented.

**The orofacial muscles and dental alignment.** By E. GWYNNE EVANS and  
W. J. TULLEY. *Guy's Hospital Medical School, London*

The relationship of the jaws, their shape and size, will determine to some extent the relationship of the dental arches, their form and the alignment of the teeth within the accommodation available to them. As the deciduous and, later, the permanent teeth successively erupt into functional position they are supported by the sponge-like bone of the alveolus which responds readily to stresses and strains imposed upon the teeth. Changes thus wrought within the alveolar bone are responsible for repositioning the teeth in the dental arches. Local forces around the teeth influence their path of eruption and there is evidence to suggest that they are carried to a position of equilibrium set up by the surrounding forces of their muscular environment. The slow-motion cine-camera has been used to record the innate behaviour patterns of the lips, circumoral muscles and tongue in all aspects of feeding and in facial expression. A film was shown to illustrate two distinct patterns of orofacial behaviour in swallowing and the types of incisor relationship associated with them.

**The Sertoli cell *in vivo* and *in vitro*.** By G. A. THOMAS.  
*Guy's Hospital Medical School, London*

Aoyama's silver impregnation technique for the demonstration of Golgi bodies has been used in an attempt to demonstrate the variations in the anatomical relationship between the Sertoli cells and the germ cells during spermatogenesis in man. This technique, as has been demonstrated in the mouse testis (Elftman, *Anat. Rec.* **106**, 1950), makes the finer branches of the Sertoli cell visible, and allows the structural details of the important relation between the Sertoli cell and the spermatid to be investigated. During spermatogenesis, variations in the structure of the Sertoli cell and in the relation of the spermatids to it occur. Fine cytoplasmic branches from Sertoli cells can be seen in contact with primary and secondary spermatocytes and spermatids. The maturation of the spermatid is associated with a considerable thickening of the Sertoli cell cytoplasm around these cells. A movement of maturing spermatozoa towards the basement membrane of the seminiferous tubule before their migration to the lumen of the tubule as they become detached from the Sertoli cell has been described in the testis of the mouse (Elftman, *l.c.*, 1950), but has not so far been confirmed in man.

Cells which have been interpreted as Sertoli cells have been observed to migrate from tissue cultures of testis. The morphology of these cells was discussed.

**Glycogen in the developing pharyngeal derivatives of the sheep.**  
By R. J. SCOTHORNE. *University of Glasgow*

The distribution of glycogen in the pharyngeal derivatives has been studied in a series of sheep embryos and fetuses from 7 mm. to approximately full term. (Fixation: alcohol-formalin-picric-acid. Staining: periodic acid-Schiff and haematoxylin. Diastase controls.)



In the 7 mm. embryo, glycogen is sparse in the pharyngeal endoderm, and is absent from the thyroid bud. By contrast it is abundant in the epithelium of the oesophagus, tracheal groove and stomach. The findings at subsequent stages are as follows:

*Parathyroids III and IV*: glycogen absent at all stages up to latest examined (110 mm.).

*Thyroid*: glycogen absent at all stages including full-term fetus.

*Thymus III*: glycogen present in endodermal component from earliest differentiation (12 mm.) to latest stage examined (340 mm.). From about 60 mm. stage onwards, glycogen is confined to the medulla, and at 340 mm. is present only in the Hassall's corpuscles.

*Cervical vesicle*: the development of the cervical vesicle in the sheep from the ectodermal vagal placode was described in a previous communication (Scothorne, *J. Anat., Lond.*, **84**, 1950). The placodal ectoderm is sharply demarcated from the surrounding skin ectoderm at the 12 mm. stage by the complete absence of glycogen. By 17 mm. the vesicle shows small quantities of glycogen, which increase further following the incorporation of the vesicle in the head of the thymus.

*Ultimobranchial body*. From 17 to 60 mm. stages glycogen is abundant in the cells of the ultimobranchial body, which differ strikingly in this respect from the surrounding thyroid tissue. Subsequently the ultimobranchial body forms a thin-walled cyst and the glycogen content of its cells is progressively reduced. At full term this cyst is lined largely by stratified squamous epithelium, but in places by simple cuboidal epithelium indistinguishable from that of the surrounding thyroid tissue. In the latest stages a few small follicles identical with those of the thyroid appear to arise directly from some parts of the cyst wall.

#### **The anatomy and embryology of the Treacher Collins syndrome.**

By J. McKENZIE. *University of Aberdeen.*

This congenital abnormality, first described in the living subject by the ophthalmologist Treacher Collins in 1900, shows notching of the lower eyelids, drooping of the outer canthi, absence of zygomatic bones and arches and deformities of the external and middle ear. The condition of the bones and soft tissue associated with this deformity in a two-months-old specimen is described to show the development errors more accurately than by clinical and X-ray examination.

A study of the arterial abnormalities in the affected regions calls for a reconsideration of the standard description of the development of the arteries derived from the first aortic arch.

#### **The role of the sacro-iliac joint in the growth of the pelvis.**

By T. J. HARRISON. *The Queen's University, Belfast*

It has been suggested that the sacro-iliac joint moves cranially during pelvic growth in the pig (Payton, *J. Anat., Lond.*, **69**, 1935). This has been studied histologically in the rat, guinea-pig and rabbit by means of serial sections, and also autoradiographically, and radiologically following the insertion of pins and wires in the pelvic bones. It is demonstrated that a cranial movement of the joint, relative to the ilium, occurs during growth in these animals also. It is shown histologically that the superior sacro-iliac ligament is made up of three sets of fibres, namely, (1) a strong upper set passing from the sacrum to the growth cartilage of the iliac crest (2) a middle set passing more directly between the two bones, and (3) a lower set in process of being detached from the ilium by osteoclastic activity. Removal of the growth cartilage on the iliac crest on one side in rats produces pelvic asymmetry during subsequent growth the acetabulum on the operated side remaining at a higher level than on the normal side. It is concluded that during pelvic growth the innominate bones on each side slide down on the sacrum because of the firm attachments of the strong upper fibres of the superior sacro-iliac ligaments. This movement preserves the normal proportions of the pelvic brim during growth. Examination of serial histological sections through this region in human foetuses suggests that a similar process occurs during pelvic growth in man.

**Effects of cortisone on the neonatal rat.** By E. J. FIELD. *University of Bristol*

Neonatal rats treated with 0.25–2.5 mg. of cortisone acetate fail to gain in weight (or actually lose weight), show greatly diminished hair growth and well-marked exophthalmos (Field, 1954). In addition, a disordered eruption and growth of incisor teeth has been noted. Within a few days of the injection the incisors appear more prominent than those of litter-mate control animals and soon become irregular in form under the influence of attrition. Later, if the effect of the injection or injections has been severe enough to cause a 50 % or more loss in presumptive weight, the incisors become curved and tusk-like, the upper ones sometimes turning upwards to perforate the palate. Such deformities seem to depend in part at least, upon a failure of attrition due to inaccurate incisal margin opposition (Prof. A. I. Darling). The general appearance of such dwarfed animals suggests pituitary deficiency associated with under-development of the jaws. Histological examination of the pituitary of cortisone-dwarfed animals shows considerable diminution both in numbers and in staining intensity of acidophiles, smallness of the cells and delayed differentiation of basophiles. Some difficulties in staining of pituitary chromophiles will be discussed. Tooth deformity has not, however, been recorded in the six-day hypophysectomized rat (Walker *et al.* 1950). Profound changes in the lymphatic system and thymus have been observed. Hassall's corpuscles are enlarged and often crowded with bizarre fragments of nuclear debris (observations on rabbits).

The appendix of cortisone-treated rabbits showed well-marked accumulations of bacteria-laden macrophages in the centres of the lymphatic nodules, but examination of trypan blue vitally stained animals showed no significant difference in the dye uptake in treated animals as compared with equivalently dosed controls.

**Observations on bladder mucosa transplants and heterotopic bone formation.**

By F. R. JOHNSON and R. M. H. McMINN. *University of Sheffield*

Several observers have noted the formation of heterotopic bone in dogs, rats and guinea-pigs following the transplantation of bladder mucosa (autografts) into the rectus sheath. The present workers have confirmed these findings in the dog, and in a series of experiments on cats it has been found that similar results are produced. Further, the induction of heterotopic bone formation has followed the transplantation of homografts as well as of autografts, despite the degeneration of the homograft itself. The various stages in the formation of this bone have been studied histochemically, with the object of elucidating some of the factors necessary for the induction of heterotopic bone formation.

The epithelium of both autografts and homografts forms cysts; the initial stages in the formation of these cysts are brought about by migration of the epithelial cells, with later proliferative activity.

**Electromyographic records and their interpretation.** By J. JOSEPH.

*Guy's Hospital Medical School, London*

While studying muscles and their actions by means of the electromyograph, several difficulties have frequently occurred. First, if the machine itself has a high 'noise level' it produces potential which may be so large that they obscure small potentials produced by the muscles or structures studied. In this connexion interference from outside sources can also obscure important potentials. It has therefore been necessary to reduce the 'noise level' as much as possible and use a screening cage. One is tempted to reduce the noise level by reducing the range of frequencies to which the amplifier responds, but it is important to ensure that the range is sufficiently wide to include all important potentials.

Secondly, the machine must be capable of high amplification so that small potentials can be seen. It is easy to demonstrate apparent absence of potentials from an actively contracting muscle if the amplification is sufficiently reduced.

Lastly, if a high amplification is used, it may be difficult to determine the source of the potentials. Circumstantial evidence may then have to be used to determine which muscle is really active, e.g. small potentials can be picked up from the tibialis anterior in a standard 'stand at ease' position. Since they *increase* if the subject sways *forwards* at the ankles and since they are *smaller* than the potentials produced by the least voluntary contraction made by the subject, it may be assumed that they originate in the soleus which can be shown to be constantly contracted in the same position. Ordinary needle electrodes do not solve this problem since it can be shown that their range of 'pick-up' varies with the subject. Records from tibialis anterior using surface and needle electrodes were shown to demonstrate this.

#### **Variations in the blood supply of the jejunum. Results of a combined survey.**

By T. E. BARLOW. *University of Durham*

Mention was made at a past Meeting of the Society (November 1953) of certain variations in the blood supply of the jejunum which in surgical procedures might be of considerable importance. The operations concerned involve division of jejunal arteries close to their origin from the superior mesenteric artery, prior to utilizing the jejunum thus mobilized to replace the oesophagus. The blood supply of the mobilized jejunum is then dependent upon the arcade system of mesenteric vessels. In certain instances it was observed that the arcade system was deficient, or that the lateral linkages were so small as to jeopardize the viability of the jejunum after operative procedures.

The active co-operation of many departments has enabled the presentation of the following figures. Of 257 cadavers examined, of all ages and sexes, 201 (78.6 %) showed a normal arcade pattern as described in the anatomical text. A further 41 (15.7 %) showed weak linkages between adjacent jejunal arteries which might not be able to carry sufficient blood to maintain viability proximally. Fifteen specimens (5.7 %) have a complete absence of anastomotic links between two adjacent jejunal arteries. The majority of absences are between first and second or second and third branches of the superior mesenteric artery. Other interesting variations are described.

#### **On the so-called 'Polypoid formations' in arteries. By D. B. MOFFAT.**

*University College, Cardiff*

During a study of human posterior ciliary arteries, a number of vessels were found containing structures closely resembling so-called polypoid cushions. In serial sections, however, these were found to occur at the point where the vessel had been divided during excision of the block of tissue from the body, the media rolling up and being invaginated along the lumen for a considerable distance.

In a series of experiments upon rat, rabbit and human tissues, structures apparently resembling polypoid cushions in every respect could frequently be found in arteries which had been divided. Similar structures could be produced at sites remote from the cut end of the artery by gentle squeezing with forceps before fixation. There is therefore a possibility that many of the structures described in the literature are artefacts, and, in particular, it is stressed that observations which are not based on serial sections are valueless.

#### **Subcutaneous homografts of the xiphoid cartilage in the guinea-pig.**

By P. BACSICH and G. M. WYBURN. *University of Glasgow*

In the guinea-pig appositional growth of the xiphoid cartilage continues in adult life. It can therefore act as a homograft for comparison of the behaviour of immature and mature cartilage. In the present experiment xiphoid cartilage was inserted subcutaneously in five guinea-pigs and grafts were removed for histological study after 21 days.



The growing more cellular immature cartilage of the graft appears to be in better histological condition than the mature cartilage with fewer cells, abundant ground substance and low metabolism.

In a previous work (1947) it was concluded that the survival of cartilage and corneal homografts was related to the mucopolysaccharide content of the ground substance. Peer (1954) and others have correlated survival with low cell content, abundant ground substance and low metabolism.

These results demonstrate the survival of growing cartilage with a high cell content, small amount of ground substance and high metabolic turnover.

#### **Cortical zonation and survival of chromaffin cells in adrenal autografts.**

By R. E. COUPLAND. *University of Leeds.*

It is commonly stated that the survival of adrenal grafts depends upon the integrity of the subcapsular cells of the zona glomerulosa and that the inner part of the cortex and medulla degenerates within a few days of transplantation.

In the present work adult rabbits and rats have been used; a thin whole slice of adrenal gland has been taken from the middle of the organ and cortex and medulla separated as accurately as possible by a knife cut along the cortico-medullary boundary. The medullary portion was then washed with isotonic saline and inserted into the anterior chamber of the left eye. In 90 % of cases the graft survives and contains chromaffin cells, together with a few cells from the zona reticularis and possibly the inner fasciculata.

Some cellular degeneration affects both cortical and medullary elements during the initial stages but is associated with proliferation of cortical elements. In some cases a typical zona glomerulosa forms around the periphery of the graft. Within 12 hr. of the removal of the remaining adrenal gland there is a marked increase in the vascularity of the implant which is followed by hypertrophy of the cortical tissue.

Some of the transplanted chromaffin cells persist in the grafts for as long as 6 months and still exhibit a well-marked positive chromaffin reaction. Mitotic figures have not, however, been observed in these elements.

#### **Cysts of developmental origin in the oral cavity of human foetuses.**

By J. H. SCOTT. *The Queen's University, Belfast*

In human foetuses of more than 100 mm. c.r. length small cysts have been found in various parts of the mouth cavity. They originate by the proliferation of isolated groups of epithelial cells. The origin of these cyst-like structures from the cell masses appears to be related to the increased vascularity of the adjacent mesoderm. In the material examined the cysts fall into three groups: (a) those of dental origin, (b) those related to the sites of union of embryological processes, (c) those arising from the epithelium of ducts and glands. Special attention has been given to a group of cysts occurring in the mid-line of the palate and reaching their greatest development in foetuses between 150 and 200 mm. c.r. length. It is suggested that these may play a part in the aetiology of perforated and cleft palates.

#### **The broncho-pulmonary segment concept applied to lungs of the Artiodactyla and Carnivora.** By D. BROWN. *Charing Cross Hospital Medical School, London*

The concept of broncho-pulmonary segments with regard to the human lung has been in use for many years, and since the Thoracic Society's Sub-Committee report of 1949 has provided a universally understood and unambiguous means of identifying any zone. The human lung, however, lends itself readily to this type of description because each main bronchus divides early in its course and the bronchial segments are few in number.

Unmodified application of similar principles to other mammalian lungs leads to confusion: first, because there is insufficient agreement concerning the names of the anatomical lobes,

and secondly, because the basic arrangement in many mammals (sheep, seal, dog) where the lower lobe bronchus has four sets of branches down much of its length, leads to the identification of a very large number of small broncho-pulmonary segments.

The essential features for comparison of the bronchial anatomy of various animals were discussed.

**Observations on the structure and development of the capsule of Meissner's corpuscle.** By N. CAUNA. *University of Durham*

Meissner's corpuscle is usually classified as an encapsulated receptor. The description of the capsule and its origin is, however, very controversial, and its existence is questioned or even denied throughout the literature.

The present investigation is based on a study of human digital skin from 107 individuals, ranging in age from the last foetal month to 93 years. Cytological, histochemical and nerve-staining methods were used. Meissner's corpuscles were compared with other receptors of the skin and mucous membranes.

It was found that Meissner's corpuscle is surrounded by a capsule of adventitial type derived from the tissue of the dermal papilla. This capsule consists of (a) cells of fibrocyte type, and (b) fine branching and interlacing elastic fibrils arranged mainly parallel to the long axis of the corpuscle. Above (towards the skin surface) these elastic fibres are interlocked with the basal projections of the epidermal cells and so support the attachment of Meissner's corpuscle to the epidermis; below they become continuous with the general elastic network of the corium. The capsule is only loosely connected to the surface of the corpuscle. The differentiation of the capsule starts after the first year of life and takes several years. The capsule does not disappear in old age while Meissner's corpuscle persists, nor during the degeneration of nerves. The other receptor with a comparable elastic capsule is the hair.

The so-called Timofeev's apparatus of fine non-medullated nerve fibres was not observed within or immediately around the capsule of Meissner's corpuscle.

**The relation of the Schwann cell to the nerve fibre.** By G. CAUSEY and H. HOFFMAN. *Royal College of Surgeons of England*

In examining with the electron microscope nerve cells and their peripheral processes, certain points have presented themselves concerning the relation of nerve fibres to Schwann cells in normal and regenerating nerve.

In normal myelinated fibres the relations are obscured by the relatively large size and great density of the myelinated fibre in relation to the Schwann cell. But in regenerating fibres the small myelinated fibres can be seen passing through the cytoplasm of the Schwann cell.

With normal non-myelinated fibres small bundles of these fibres are found, each individual fibre being surrounded by a double membrane, but in addition the whole group is surrounded by a double membrane and evidence will be produced that this encircling double membrane is the membrane of the Schwann cell and that cytoplasmic inclusions can be demonstrated outside the nerve fibres and inside the cell membrane.

In regenerating nerve when the Schwann cells are more numerous the small fibres, before myelination, can be seen outside the nuclear membrane of the Schwann cell but inside the cell membrane.

**The phrenic nucleus of the rhesus monkey.** By R. WARWICK and G. A. G. MITCHELL. *University of Manchester*

The phrenic nerve of rhesus monkeys was shown in a previous communication to be derived from the third to the sixth cervical spinal nerves (chiefly the fourth and fifth). The regular production of marked retrograde degeneration in the motor cells of the

phrenic axons, caused by divisions of the nerve even as distant from its origin as the diaphragm, was also demonstrated. The precise distribution of these degenerative effects has now been analysed in serial sections prepared from twenty-three animals subjected to phrenic division at a variety of levels in the neck and thorax.

This analysis shows that the motor pool of the phrenic nerve is a topographically discrete column of nerve cells situated in the ventral zone of the ventral grey column and extending for approximately 10 mm. in cervical segments three, four and five.

Although this columnar mass may be regarded as a nucleus, its constituent nerve cells show a clear arrangement into little globular clusters, separated by intervals devoid of motor neurones.

No evidence that any phrenic axons decussate before emergence from the cord was observed, but interruption of the right phrenic nerve alongside the inferior vena cava caused chromatolytic changes in the left as well as in the right phrenic nucleus. This indicates an exchange of fibres between the phrenic nerves, presumably at pericardial levels, since interruptions of the phrenic trunk, left or right higher in the thorax, produced no such bilateral effects.

**A statistical study of the *boutons terminaux* of the cervical region of the spinal cord of the cat.** By G. W. PEARCE (*University of Dundee*) and P. GLEES (*University of Oxford*)

At the 1954 meeting of the Anatomische Gesellschaft, Pearce & Glees classified the *boutons terminaux* of the third and sixth cervical segments of the spinal cord into five varieties, thin, thick, reticulated, opaque and fragmented, and gave evidence that this classification may serve as a basis for statistical studies of normal and degenerated *boutons*. The paper is an extension of the above work and part of a morphological and statistical study of the *boutons* in the spinal cord and brain stem.

In six cats we measured the longest and shortest diameters of the *boutons* and also calculated the percentage of the five varieties of *boutons* upon the cells of the antero-lateral, postero-lateral (C6 only), medial and intermedio-medial areas.

The following are the chief results:

- (1) Reticulated *boutons* have twice the area of thin.
- (2) The internuncial cells of the medial and intermedio-medial areas have the same proportions of *boutons*.
- (3) The motor cells of the antero-lateral area of C3 supplying chiefly postural muscles have a statistically significant higher proportion of reticulated and a lower proportion of thin *boutons* than the motor cells of the antero-lateral and postero-lateral areas of C6 supplying the voluntary muscles of the upper limb.
- (4) The motor cells of C3 have the same proportion of all *boutons* as the internuncial cells, but there are only between two-thirds and one-half as many reticulated *boutons* upon the motor cells of C6 as upon the internuncial cells of C6.
- (5) Thus, it is suggested that thin and reticulated *boutons* have separate functions.

**Fibre degeneration following lesions of the hippocampus-fornix system.**

By T. P. S. POWELL and W. M. COWAN. *University of Oxford*

Lesions were placed in various parts of the hippocampus-fornix system in rats, rabbits and monkeys and the resulting fibre loss studied in sections stained with Bodian's activated protargol. Division of the fimbria and dorsal fornix results in complete atrophy of the descending column and of the pre-commissural fornix. After interruption of the fimbria alone in rats and rabbits there is complete degeneration of the pre-commissural fornix and a slight reduction in diameter of the descending column at the level of the mamillary nuclei. Division of the dorsal fornix alone in these species produces no change in the pre-commissural system but results in a marked fibre loss in the descending column. From these



observations it is concluded that most of the efferent fibres of the fimbria end in the hypothalamus rostral to the mamillary nuclei and that most of the fibres which do reach the mamillary nuclei are derived from the dorsal fornix. A proportion of the fimbrial fibres which end in the anterior hypothalamus leave the descending column in the medial cortico-hypothalamic tract. but, in addition, a considerable number have a more diffuse distribution.

## FEBRUARY 1955

An ordinary meeting of the Society for the Session 1954-5 was held on Friday, 18 February 1955, at the Department of Anatomy, Royal Free Hospital School of Medicine, Hunter Street, W.C. 1. The President (Prof. W. J. HAMILTON) was in the Chair.

The following are the authors' abstracts of the papers read.

### **The relations of the recurrent laryngeal nerve in the neck.** By RUTH E. M. BOWDEN. *Royal Free Hospital School of Medicine, London*

The relations of the recurrent laryngeal nerve in the neck are of importance in the surgery of the thyroid gland. Since the variations are so numerous it is difficult to find a precise and comprehensive account. The present observations are therefore correlated with those published in the literature in the hope of clarifying points of surgical significance. The materials used include nineteen dissecting room cadavers, nine fresh post-mortem specimens, two preserved specimens, and a departmental record of an anomaly of the right recurrent laryngeal nerve. A specimen consisting of the head, neck and upper part of thorax of a 7 months' foetus which had been fixed by injection of 10 % formol-saline was decalcified, sectioned serially at  $40\mu$  and stained. Particular attention was paid to the relevant parts of the cervical fascia and to the relations, frequency and site of branching of the recurrent laryngeal nerves as well as to certain vascular anomalies.

### **Prehensile movements of the human hand.** By J. R. NAPIER. *Royal Free Hospital School of Medicine, London*

Although prehensile movements of the hand as a whole are among the most highly specialized in the body, recourse must be made to such non-specific terms as pinch, grip and grasp, in order to describe them; these terms have little scientific meaning.

At first sight it would seem that prehensile movements of the hand would, by their very profusion, defy analysis. Yet this diversity is in fact not an expression of a multiplicity of movements, but of the vast range of purposive actions embodying objects of all shapes and sizes that are an essential part of everyday life. A study of the normal and the abnormal living hand suggests that there are in fact only two distinct action patterns in man, which either separately or in combination provide the anatomical basis for all movements, whether skilled or unskilled. It is suggested that these movements should be termed 'precision grip' and 'power grip'.

### **Studies on the afferent nerve supply of the facial muscles.** By Z. Y. MAHRAN. *Royal Free Hospital School of Medicine, London*

In view of the delicate control of facial expression in man, it has been presumed that there is an afferent nerve supply to the facial muscles. One suggestion, for which no evidence has been adduced, is that the sensory fibres might reach the VIIth nerve through the communications with branches of the Vth nerve.

So far the nature of these communications has not been investigated and spindles have not been reported in facial muscles.

The rich communications between the VIIth nerve and the Vth nerve, well known in man, have been reinvestigated by dissection. They have also been found in the cat, rabbit and the common grey seal.

In the rabbit, histological studies in the normal have shown that the communications consist of fasciculi of myelinated nerve fibres. After experimental operations it was shown that these fasciculi pass from the Vth nerve into the VIIth nerve. The fasciculi may remain separate, or form more complex intraneural communications.

Simple spindles have been found in the facial muscles of the rabbit.

**Observations on the movements of the human lower thoracic and lumbar vertebrae.** By P. R. DAVIS. *Royal Free Hospital School of Medicine, London*

A study of the human thoraco-lumbar transitional region was described in a previous communication. It was shown that the region of functional transition was commonly marked by a neural arch joint resembling a carpenter's mortice. It was suggested that the mortice joint is the least mobile joint in that region.

Experiments on osseoligamentous preparations are described, which have shown:

(1) That antero-posterior movement is reduced in the transitional region, but is not always least at the mortice joint.

(2) That axial rotation is usually least at the mortice joint.

(3) That given a sufficient moment of rotation, considerable axial rotation can occur in the lumbar region, as well as in the thoracic region.

An experiment was also described which showed that rotatory moments considerably in excess of those used in the experiments can be produced by the trunk musculature in the living.

Dr C. H. Barnett, of St Thomas's Hospital, has obtained radiological evidence that voluntary axial rotation of a considerable degree does occur in some individuals, and the writer is very grateful for his permission to publish his results.

**Some factors in the maturation of the genital tract of the female kitten.**

By PATRICIA P. SCOTT. *Royal Free Hospital School of Medicine, London*

The occurrence of oestrus at an early age (5 months), followed by normal pregnancy, was observed in some female cats which had received a standard high-protein diet, supplemented with penicillin, subsequent to weaning. A preliminary investigation of the factors concerned in this early maturation was carried out on a further sixteen female kittens, approximately 10 weeks old, which had the same male parent. These were arranged in two balanced groups, one group being given the standard diet alone, the other receiving the diet supplemented with 30,000 i.u. penicillin G per kg. of wet diet. Some of the kittens in each group were given 10 mg. implants of oestradiol, while one animal received an implant of 50 mg. of testosterone propionate, for the duration of the experimental period of 6 weeks. Penicillin added to the diet brought about an increase to the weight of the tract of kittens without implants, greater than that to be expected on the basis of the increases in total body weight. Gross and histological changes were described.

**The value of the vaginal smear technique in following the reproductive cycle of the female cat.** By MARNY A. LLOYD-JACOB and PATRICIA P. SCOTT. *Royal Free Hospital School of Medicine, London*

The behaviour of cats in the colony at this Institution has been studied over a period of 2 years, during which time over 2000 vaginal smears have been collected from normal animals. The domestic cat is polyoestrous, the length of the cycle being approximately 14 days with the period of oestrus lasting from 4 to 6 days, although it may be prolonged

by lack of service. Oestrous behaviour is characteristic and easily recognizable, and during oestrus, mating occurs several times a day for 3 or 4 consecutive days. Vaginal smears from each stage of the cycle will be described and correlated with appropriate sections of the vaginal wall. The examination of vaginal smears has proved a reliable and convenient method for the recognition of the various phases of the reproductive cycle, provided that smears are taken correctly and fixed immediately without drying in air.

**Some preliminary observations on the minute structure of the lower birth canal in the rabbit.** By F. J. AUMONIER (introduced by K. J. FRANKLIN). *St Bartholomew's Hospital Medical College, London*

Since previous accounts of the fine structure of the lower genital tract in the rabbit are somewhat lacking in detail it was considered useful to study this region.

The material used consisted of a series of young female rabbits of the Rahere strain. The animals were killed without any special reference to the date in the oestrous cycle, and the junction of the stratified and columnar types of epithelium was studied. In addition, the arrangement of the muscle coats was examined.

The vestibule which extends from the vulva to the entry of the urethra is lined with stratified squamous epithelium. Slightly above this point the epithelium is of simple columnar type. The muscle coat consists at first of a longitudinal layer of smooth fibres. Approximately 20 mm. within the vulva a spiral coat of smooth muscle appears. About 15 mm. above the entry of the urethra an outer longitudinal layer is found. Both spiral and outer longitudinal coats interlace in the vicinity of the cervixes.

**Blood supply of the mystacial vibrissae of the rat and cat.** By M. G. SCOTT (introduced by P. P. SCOTT). *Royal Veterinary College, London*

Injection and histological techniques have revealed the following features in the blood supply:

(1) An artery penetrates the outer dermal sheath of the follicle with the sensory nerve, passes across the middle dermal sheath and divides into branches which ultimately give rise to the capillaries of the hair matrix and to those associated with the outer root sheath. Before entering the outer dermal sheath this artery gives branches to the associated striated muscles and to a superficial plexus surrounding the necks of follicles.

(2) The venous drainage is apparently by minute vessels arising from the region adjacent to the hyaline membrane which unite to form a single vessel, still of capillary dimensions. This passes through the outer dermal sheath, suddenly dilates and joins a vein into which the efferent vessel from the dermal papilla also drains. The unusual arrangement of the afferent and efferent vessels supports the view that blood is always present in the sinus system. Contrary to previous descriptions the sinus tissue is not erectile in character.

(3) The dermal papilla is supplied by a minute afferent vessel derived from the capillary plexus of the hair matrix. It gives rise to multiple branches dividing up into a network of capillaries which cover the surface of the papilla, some ascending in twisting loops to its apex. The capillaries drain into a central efferent vessel of larger calibre which leaves the base of the follicle and joins the large vein mentioned above.

**The mechanics and function of endarthrodial plates.** By M. A. MACCONAILL. *University College, Cork*

Barnett (1954) has explained the presence of fibro-cartilages and menisci within synovial joints by associating them with marked gliding movement at the joint; he states that this explanation is not very satisfactory for the carnivorous jaw joint. His explanation is, therefore, a kinematic one; only two variables are involved, namely, the presence (or absence) of endarthrodial plates and the presence (or absence) of gliding movement.



There are two other variables which can be brought into this problem. One of them is the form of the articulating extremities of the bones included in the joints; the other is the strength of the transarticular thrust. There are thus two 'structure elements' and two 'function elements'. It is shown that this leads to a fourfold classification of structure and a fourfold classification of function.

If there is a one-one correspondence between structure and function then this can be arrived at only by invoking an existing law, namely, that the absence of an endarthrodial plate is incompatible with the conjunction of strong articular thrust, and any type of articulation except that in which the articulating surfaces are not at once well curved and nearly congruent in most positions (MacConaill, 1932, 1944, 1950). This solution is explicitly kinetic and it accounts for the structure of the carnivoran jaw joint. In so far as Barnett's law is a function of two variables only it is secondary to the kinetic law, which contains four variables.

### **Skeletal remains from an ancient burial mound in County Down.**

By W. R. M. MORTON and J. H. SCOTT. *Queen's University, Belfast*

A remarkably large and relatively well-preserved collection of unburnt human bones and teeth, and some incinerated remains from a previously undisturbed main burial chamber of a Neolithic or Early Bronze Age grave at Millin Bay, County Down, are briefly described. Fourteen to fifteen individuals are represented, and examples of every bone of the body have been identified with the exception of the sternum, coccyx, some carpal bones, terminal finger phalanges and the hyoid. Moderately complete skeletons have been re-assembled and an impression of family likeness is given by the material. The skulls and mandibles are imperfect, and only approximate indices could be derived. Incorrect replacement of two incisor teeth in a maxilla and a mandible found to be present at the time of opening the grave, associated with the piling of the bones in a heap, indicates that reburial had occurred at the time of burial in the chamber. An intact right humerus and left radius are the only upper long limb bones from which exact measurements could be taken, but two almost complete femora and a tibia, and several other lower limb bones, have been sufficiently well preserved for measurements of their length to be estimated. Many small bones are intact or almost so, and a large number of bones with their ununited epiphyses have been found. Four or five adults, six young adults and adolescents, and four children between about 6 and 12 years of age appear to be the minimum numbers present. Four burials of incinerated human material in small cists have been found apart from that in the main burial chamber. Four, or possibly five, adults are represented by this material.

### **Experimental observations on respiration through the humerus of *Gallus domesticus*.**

By P. M. BIGGS and A. S. KING. *University of Bristol*

The fact that some birds can breathe through the transected humerus with trachea ligated was demonstrated by John Hunter. Seven experiments have been done to test this in *Gallus domesticus*. In six, humeral respiration was successful to a varying degree for varying periods, the maximum period being 3 hr. 41 min. In the remaining one, duration was only a few seconds. In some experiments ventilation and oxygen consumption were determined for both tracheal and humeral routes. Comparison of these values showed that the abnormal route always changed the pattern of respiration to a greater or lesser extent.

Factors possibly responsible for this change were:

- (1) Compression of the humeral diverticulum of the interclavicular sac.
- (2) Blood clot inside the humerus.
- (3) Anaesthesia.
- (4) The size of the airway through the humerus.
- (5) The dead space of the interclavicular sac.
- (6) Disturbance of the normal circulation of air in the lung.

The first three should be controllable by correct technique. Measurements suggest that (4) should be insignificant. Direct experiments to estimate the effects on respiration of (4) and (5) was discussed.

If these first five factors can be controlled it may be possible to evaluate the sixth, the effect on the lung circuits. Humeral respiration may therefore offer a means of testing Hazelhoff's (*Poultry Science*, 30, 1951) *d-p-v* concept of air circulation within the avian lung. This possibility was discussed.

**Observations on nerve endings in Meissner's corpuscle.** By N. CAUNA.  
*King's College, Newcastle upon Tyne*

The manner of nerve termination in Meissner's corpuscle has been the subject of extensive controversy for a century. Descriptions have varied from a denial of intra-corporcular nerve endings (Kölliker, Huxley) to an assurance that the neurofibrillar apparatus formed continuity with the cytoplasm of the receptor cells (Boeke).

This investigation is based on human material from 102 individuals ranging in age from birth to 93 years. The age, sex and occupation have been considered in assessing the material.

It was found that Meissner's corpuscle was usually supplied by 3-4 axons derived from different nerve bundles of the corium. Each axon divided repeatedly into branches within the corpuscle, but sometimes before reaching it. The myelin sheath was always lost before a nerve fibre entered the corpuscle, but lemmocytes and even the cells of the endoneurium were found in the periphery of the corpuscle. The lower (corial) part of Meissner's corpuscle consisted of modified lemmocytes.

Nerve endings formed horizontal layers between the flattened cells of Meissner's corpuscle. In infants, the dominant type of termination consisted of closed end-bulbs and open neurofibrillar networks. Later in life four main types of nerve ending were observed: (1) closed end bulbs; (2) open neurofibrillar networks; (3) series of varicosities in the intra-corporcular part of the nerve fibres; (4) simple terminal ramifications.

In manual workers a considerable reduction of neurofibrillar apparatus was observed. In old age the nerve endings were restricted to the upper part of the corpuscle, the lower part consisting of twisted bundles of ascending nerve fibres.

**Experiments on the development of the interorbital septum in chick embryos.**  
By A. d'A. BELLAIRS. *St Mary's Hospital Medical School, London*

In birds there is a thin plate of bone between the eyes known as the interorbital septum. It originates in the embryo from the fusion of the paired trabeculae and orbital cartilages with a third median element, regarded as an intertrabecula, the whole being compressed into a thin vertical plate. It has been suggested that an essential factor in the development of the septum is the mechanical pressure of the very large eyes.

To test this theory, removal of one optic cup in chick embryos of 2-3 days has been performed and the embryos incubated for various periods, fixed, sectioned and compared with normal controls. Study of seven operated embryos which have developed successfully shows that the process of fusion of the different components of the septum is unaffected by the experiment, which was performed about 48 hr. before the first rudiments of the skull become visible.

**Observations concerning the position and relations of the recto-vesical and recto-vaginal pouches in the human embryo and foetus.** By P. H. S. SILVER.  
*Middlesex Hospital Medical School, London*

In the 4 months human foetus the recto-vesical pouch has been described by Cunéo & Veau and by Elliott Smith as lying below the adult position. Zygosis at the bottom of the pouch has been said to occur, resulting in its upward migration. Anteriorly, at this stage,

the pouch is seen to lie opposite the upper part of the prostate, and the whole of the posterior surface of the bladder is covered by peritoneum. But when the posterior relations are considered (they do not appear to have been taken into account by previous workers), the pouch is seen to lie in the region of Houston's main valve, i.e. in the definitive adult position. These apparently contradictory observations have prompted this study of the recto-vesical and recto-vaginal pouches.

Thirty human embryos and fetuses have been examined. Observations have been made showing the relations of the pouch to the adjacent structures and the distance from the surface of the perineum and pelvic skeleton. The evidence does not support the simple interpretation of Cunéo & Veau, but indicates the importance of differential growth processes affecting the pelvic viscera.

**The fibres in dentinal tubules.** By M. A. MACCONAILL. *University College, Cork*

Potassium permanganate forms a deep blue insoluble lake with methylene blue (solution pH 10). The odontoblast fibres have a special affinity for the permanganate, and so the alkaline methylene blue can be used to colour them intensely and to trace their course. The fibres can be traced in continuity from the odontoblast through the meshes of the pulpo-dentinal membrane and thence to the pre-dentine and dentine proper. Alkaline methylene blue without  $\text{KMnO}_4$  stains the wall of the tubule and enables it to be distinguished from the dentinal fibre. The form and branching of the dentinal fibres were described. The demonstration also showed the form and branching of the tubules proper.

**A peritoneal specialization in the squirrel monkey (*Saimiri*).**

By W. C. OSMAN HILL. *Zoological Society of London*

The short, straight hind-gut of *Saimiri*, with the caecum often directed cranial from its proximal end, has been regarded, since first described by Martin (1833), as a primitive feature, inasmuch as the arrangement shows no advance on that in *Tupaia*. Although there is no gainsaying the relatively undeveloped state of the gut, this cannot be accepted as regards its peritoneal relations.

On opening the abdomen and raising the great omentum, a huge peritoneal sac is revealed with all the intestines inside it. This at first sight suggests some form of internal or retroperitoneal hernia, but its constancy proves it to be a normal feature in this genus.

The sac is produced by a dextral rotation of the hind-gut upon its own axis, coupled with a clockwise shift of the gut into a position from whence it started—except that its mesenteric wall now faces ventrad instead of dorsad. The mesocolon is thus drawn over the coils of small intestine investing them in a thin, membranous bag of pyriform shape, the narrow end towards the pelvis. The cavity of the bag is therefore a sort of lesser sac, largely shut off from the general peritoneal cavity. It may be appropriately named *bursa mesocolica*. Its boundaries and attachments were described and demonstrated.

It is remarkable that this peculiarity has escaped attention hitherto in such a commonly imported monkey, but none of the classic writers (Klaatsch, van Loghem, etc.) refer to it.

**The comparative anatomy of the secondary tympanic membrane and periotic duct.** By C. C. D. SHUTE. *University of Cambridge*

In view of differences of opinion as to the homology and attachments of the secondary tympanic membrane in mammals, the region of the recessus scalae tympani, cochlear aqueduct and round window has been investigated by means of serial sections of late embryonic stages of various reptiles and mammals.

The membrana propria in reptiles, unlike the dense band characteristic of mammals, is a late formation, but the latter may have evolved from mesenchymal strands similar to those seen in the course of development of the secondary tympanic membrane of skinks.



The shape and attachments of the mammalian secondary tympanic membrane are more complex than has been generally recognized. Anteriorly it is flat, and its medial rim is inserted into the lateral edge of the processus recessus. Posteriorly it is drawn inwards to form part of the intra-otic orifice of the aqueductus cochleae, and to close the hinder end of the foramen perilymphaticum.

In certain mammals the membrane extensively invaginates the inner ear. This is characteristic of bats, but also occurs in elephant shrews. The consequent reduction in the effective mass of perilymph may facilitate the appreciation of high-pitched sounds.

The periotic duct may have evolved as a communication between the perilymphatic sac and the submeningeal space surrounding a cranial nerve root: X in turtles, IX in crocodiles and mammals. The membranous wall of the duct forms the separate dural sheath surrounding IX in the jugular foramen. In animals with a long glossopharyngeal root, the latter may run a considerable course through the duct.

**Observations on regenerating transitional epithelium.** By R. M. H. McMINN  
and F. R. JOHNSON. *University of Sheffield*

Regeneration of transitional epithelium in the bladder has been studied in a series of adult cats. An 'artificial ulcer' was made by removing approximately 1 sq.cm. of mucosa from the dorsal wall of the bladder, and the site of this lesion was examined histologically at varying post-operative periods up to and including the stage of complete healing. Some migration of epithelial cells from the margins of the ulcer towards its centre can be seen within 24 hr. of operation. By the second day, numerous mitotic figures can be seen both in the normal epithelium adjacent to the ulcer margins, and in the single layer of cells which continues to spread over the floor of the ulcer. Similar appearances are noted during subsequent days, though the degree of proliferative activity would appear to be maximal from the second to the fifth or sixth days. A healed ulcer covered by stratified epithelium may be seen as early as the ninth day.

These findings were correlated with observations that the authors have made on the survival of autografts and homografts of transitional epithelium, and compared with healing activity in other epithelia.

**A case of sympodia.** By I. N. LEE (introduced by E. W. WALLS).  
*Middlesex Hospital Medical School, London*

The infant, 4 lb. 8 oz. in weight, was still-born to a 24-year-old primigravida in the 38th week of pregnancy. No family history of congenital malformation could be elicited. Above a large umbilical hernia the child appeared normal apart from a pre-pollex on the right hand; but below, instead of lower limbs, there was present a single central appendage which gradually tapered to a point furnished with two small nails. There was no anus or urogenital orifice, nor any evidence of external genitalia.

Dissection revealed the innominate bones to be fused, forming one posterior acetabulum bounded above by fused posterior iliac spines and below by fused ischia. A single central femur articulated with the acetabulum, and by its lower end, which possessed two secondary centres of ossification, with a single tibia in a forward flexing joint. Three digital ossification centres were present at the extreme end of the appendage. The anterior aspect of the thigh revealed bilaterally symmetrical muscles, identified as gracili, adductor masses and sartorii, together with two obturator nerves and bilateral femoral nerves and vessels. On the posterior surface of the femur there were two rectus muscles ending in two patellae. The vastus masses were fused. No flexor muscles were present, and the muscles below the knee were also lacking.

In the lower abdomen apparently normal ovaries and fallopian tubes were present, but the uterus was represented by a small solid body quite devoid of a lumen. The urinary apparatus was completely absent. Right and left adrenal glands of large size were present.

Other abnormalities discovered were: a single umbilical artery arising from the aorta; a persistent left superior vena cava; absence of communication between the pharynx and oesophagus; the presence within the umbilical hernia of part of the right lobe and of the caudate lobe of the liver, and also of the gall bladder; the gut ended blindly in the sigmoid region.

The possible mechanism of production of this exceedingly rare condition was discussed.

## APRIL 1955

An ordinary Meeting of the Society for the Session 1954-5 was held on Friday, 22 April 1955, in the Department of Anatomy, University College, London, W.C. 1. After the morning session, owing to the large number of communications, the meeting was divided into two sections working concurrently. The President (Prof. W. J. HAMILTON) and Prof. J. S. Baxter occupied the Chair at the various sessions.

The following are the authors' abstracts of the papers read.

### **The optic chiasma of cephalopods and the significance of crossed tracts.**

By J. P. STANIER and J. Z. YOUNG. *University College, London*

In cephalopods the retinal fibres undergo decussation immediately behind the eye in such a manner that fibres from the dorsal side of the retina proceed to the ventral side of the same optic lobe and vice versa. There is no comparable decussation antero-posteriorly, and this suggests that the chiasma serves to bring the visual information, inverted by the lens, into correct relations with information from phylogenetically older non-inverting receptors such as those for gravity.

The functioning of visual analysers thus seems to depend upon maintenance of certain spatial relations. If the same condition holds in vertebrates the optic decussation across the mid-line may have arisen as a means for bringing about the correct relation without the interweaving of fibres that is necessary in a unilateral decussation such as that of cephalopods.

### **Observations on the larger anterior horn cells in the lumbar region of the cat's spinal cord.**

By J. T. AITKEN, *University College, London*

The dimensions of the ventral horn cells of the spinal cord have never been determined, although they are of crucial importance in the interpretation of spinal reflex activities.

Preparation of Golgi-stained material suitable for measurement has proved difficult probably owing to the poor penetration of the osmic acid solutions. With the methods used, the great size of the cell bodies and the extent of their dendrites appears. The details of the ramifications were measured by the methods developed by Sholl (*J. Anat., Lond.*, 1953). The area of the perikaryon may be as much as  $12,000\mu^2$  and of the dendrites  $68,000\mu^2$ . Dendrites were found which ended about 1 mm. from the cell body. No axon measured more than  $5\mu$  in diameter whilst still in the grey matter of the cord.

### **The surface area of cortical neurons.**

By D. A. SHOLL. *University College, London*

The acceptance of the neuron theory, with the corollary that the surfaces of the dendrites and perikaryon form the receptive part of the neuron, implies that any discussion of the organization of the cerebral cortex or any attempt to interpret the potential differences detected by microelectrodes must consider the extent of these surfaces. No previous attempt seems to have been made to measure the dendritic surface and relate it to that of the perikaryon.

Measurements have been made on a number of neurons from the cerebral cortex of the cat in thick sections ( $150\mu$ ) stained by the Golgi method. The regions of the cortex selected for the study were from the sensori-motor and visual areas. The total volumes of the cells ranged from 8000 to  $33,000\mu^3$ .

A general survey of the results showed that the dendritic surface forms approximately 90 % of the total receptive surface of the neuron. Some of the smaller stellate cells showed a dendritic surface of about 85 % of the total, but, in general, no differences of this kind could be found between stellate and pyramidal cells. Plotting the area of the dendritic surface against perikaryal surface showed that the relationship could be regarded as approximately linear. Fitting a linear regression showed that, in general, 90–95 % of the receptive surface of the neuron was formed by the dendrites.

**Cell territories in the cerebral cortex of the rat.** By H. MATURANA  
(introduced by J. Z. YOUNG). *University College, London*

Neurone counts in different regions of the cerebral cortex were undertaken with a view to making an indirect estimation of the interneuronal plexuses. No special attention was paid to cortical areas or laminar arrangements, and Nissl preparations of transverse sections were selected at equal distances along the longitudinal axis of the brain. Parasagittal sections which passed through the frontal and occipital poles were also used. Thus in twenty-one regions evenly distributed on the cortical surface the total number of neurons, in its whole thickness, was counted. In the same regions, the nuclear and cell diameters were measured for the Abercrombie correction and calculation of cell territory.

It was found, in the six animals used, that the number of cells per unit of surface (in the whole thickness of the cortex) was nearly constant in all the parts of the isocortex examined. As the cortex increases in the thickness from the occipital to the frontal pole, the cell density decreases in the same direction and the cell territory becomes at the anterior end twice as large as at the posterior end. The cell density also decreases from the dorsal to the lateral aspect of the cortex, though less markedly.

This increase in cell territory from the back to the front and from the dorsal to the lateral aspect of the cortex is not due to an increase in the glial cells and blood vessels, since the count of their nuclei shows that their density is nearly constant throughout the cortex. The large differences in cell density in the frontal and occipital poles suggest that there may be significant differences between the number of afferent and efferent fibres and the extent and complexity of the intercellular plexuses in the predominantly motor (frontal) and the predominantly sensory (occipital) regions of the cortex.

**A study of fibre degeneration methods in the nervous system.**  
By D. H. L. EVANS and L. H. HAMLYN. *University College, London*

A number of histological techniques have been used for the investigation of degenerating nerve fibres and terminals within the nervous system. In view of the difficulties experienced by the authors in interpreting the results of some of the reduced silver methods, an attempt has been made to compare these and other techniques in situations where the neuro-anatomical connexions are being investigated.

For this purpose use has been made of the Bielschowsky-Gros, the Glees and Nauta methods. Although the three methods can be used for studying the course and site of termination of fibre tracts the appearances produced by the Bielschowsky-Gros and Glees techniques differ from those obtained in Nauta preparations.

Examples of the results of this comparison of methods, after varying degeneration times, were shown in the avian optic tectum, the mammalian neo-cortex and the mammalian medulla oblongata. The interpretation of the different appearances produced were discussed.



**Nissl's substance, cytoplasmic filaments and nuclear membrane of spinal ganglion cells.** By I. M. DAWSON and G. M. WYBURN. *University of Glasgow*

Thin sections of spinal ganglia of rabbit were examined with the electron microscope.

Spinal ganglion cells show variation in the form and distribution of Nissl's material within the cytoplasm. On the basis of this difference two types of cell can be distinguished: (a) 'light' cells where Nissl's substance appears as discrete aggregates of  $1-2\mu$  in diameter, much larger than the mitochondria ( $0.25\mu$ ). (b) 'Dark' cells where Nissl's substance is diffusely dispersed throughout the cell giving a more homogeneous and denser appearance to the cytoplasm.

The basic structural component of Nissl's substance is optically an electron dense, finely granular material of particle size,  $50-200\text{ \AA}$ .

There is a background network of fine filaments  $20-50\text{ \AA}$ , probably protein, in the cell cytoplasm and in the axon. These form the structural units in both cell and axon. They are much finer than the neurofibrillae just visible with the light microscope and show no evidence of lateral aggregation.

The thickness of the nuclear membrane varies from cell to cell and is not uniform around any single nucleus. It is, in general, from  $600$  to  $1000\text{ \AA}$ . The membrane is made up of two components: (a) a continuous separating membrane showing stratification into the seven or eight layers spaced  $100-150\text{ \AA}$  apart, probably protein layers; (b) nodes of around  $850\text{ \AA}$  in diameter, irregularly spaced at  $250-1800\text{ \AA}$ . An approximate estimate gives a value of  $10,000$  nodes on the whole membrane. In radial sections the nodes are  $1000\text{ \AA}$  in both dimensions and are interpreted as lipoprotein layers forming the bounding walls of central canals or pores, filled with diffusing material. It is suggested that the mechanism for the selective diffusion of material through the membrane is a variation in the disposition of molecular constituents affecting, in particular, the structure of lipid-lined pores.

**The function of the interstitial cells of Cajal in the guinea-pig ileum.** By J. MCKENZIE, H. W. KOSTERLITZ and JUDITH A. ROBINSON. *University of Aberdeen*

Trendelenburg (*Arch. exp. Path. Pharmac.* **81**, 1917) found that the normal reaction of the isolated guinea-pig ileum to filling, i.e. the peristaltic reflex, consists of contraction of the longitudinal layer followed by contraction of the circular layer. While storing the intestine in Tyrode solution for 24 hr. at  $4^\circ\text{C}$ . abolishes the contraction of the circular coat, it does not interfere with the contraction of the longitudinal coat, which is not inhibited by ganglion blocking agents. The response of the longitudinal muscle layer is not a mechanical shortening caused by filling of the lumen nor is it due to stretch of the longitudinal fibres themselves; the adequate stimulus is radial distension (Kosterlitz, Pirie & Robinson, *J. Physiol. Proc.* 1955).

Using the Schabadasch methylene-blue technique to stain the autonomic ganglia and fibres and the interstitial cells of Cajal, we find that in material stored for 24 hr. the autonomic ganglia and fibres, but not the interstitial cells, stain more rapidly in the methylene blue than in fresh intestine. When the reaction of the longitudinal layer itself is abolished by storage for 4-5 days in the Tyrode solution at  $4^\circ\text{C}$ ., the interstitial cells become stained in less than 10 min. as opposed to 20 min. in methylene blue for normal fresh material. The muscle fibres which still respond to acetylcholine or histamine do not stain under these conditions. Previous workers with this technique state that staining occurs when the tissue is dying or dead.

It would appear that this histological evidence regarding the viability of the autonomic and interstitial cells is correlated with the physiological reactions of the intestine, a finding compatible with the view that the interstitial cells of Cajal may be the intermediary for the contraction of the longitudinal muscle coat after the intestine has been stored at  $4^\circ\text{C}$ . for 1-2 days.

**The projection of the midline and intralaminar nuclei of the thalamus of the rabbit.** By W. M. COWAN and T. P. S. POWELL. *University of Oxford*

The efferent connexions of the midline and intralaminar thalamic nuclei have been investigated in a series of thirty-five rabbits. Stereotaxic lesions and selective cortical ablations were made in the rostral part of the cerebral hemisphere and the resulting retrograde cell degeneration in the thalamus studied after survival periods of 1-3 months. It has been established that these nuclei have extrathalamic connexions and that they project by way of the inferior thalamic radiation to the vicinity of the head of the striatum. The intralaminar nuclei centralis medialis, paracentralis and centralis lateralis project to the head of the caudate nucleus while the parafascicular nucleus is directly related to the putamen. The precise connexions of the parataenial and paraventricular nuclei could not be determined, although the former is probably connected with the nucleus accumbens. The cells of the midline nuclei reuniens and rhomboideus send their axons to the cortex of the infralimbic area on the medial surface of the hemisphere overlying the caudate nucleus.

**Quantitative observations on the mitochondria in the mammary gland of the guinea-pig.** By A. HOWE and K. C. RICHARDSON (*University College, London*) and M. S. C. BIRBECK (*The Chester Beatty Institute of Cancer Research, London*)

The respiratory quotient for mammary tissue is well below unity at the end of pregnancy. In homogenates and mitochondrial fractions of guinea-pig mammary gland the succinic dehydrogenase and cytochrome oxidase activity increases seven- to nine-fold when the mammary alveolar epithelium is actively secreting after parturition (Moore & Nelson, *Arch. Biochem. Biophys.* 36, 178, 1952). Some quantitative estimations of the mitochondria in alveolar cells from lactating and non-lactating glands have been made from stained paraffin sections and from electron micrographs. Sections taken at random indicate that the average amount of mitochondrial substance per unit area of cytoplasm remains constant in the two phases of the gland. The cells, however, increase in average sectional area about two-fold, and this cell hypertrophy, which takes place in the course of a few days, must therefore be accompanied by a corresponding increase in the amount of mitochondria. It is unlikely that the acceleration of cell activity is accompanied by any disproportionate increase of mitochondrial substance per unit volume of cytoplasm. In the past it has been commonly stated that mitochondria are of even diameter in individual cell types, varying only in length. Detailed measurement of a large number of sectioned mitochondria in the electron micrographs indicates that they are not uniform but probably range in thickness from about 0.15-0.55 $\mu$ .

**Certain features of the anatomy of the anal canal.** By E. W. WALLS.  
*Middlesex Hospital Medical School, London*

Examination of longitudinal sections through the lower part of the rectum and through the whole length of the anal canal reveals the following points:

(1) An anal intermuscular septum as described by Milligan & Morgan (1934), and since that time widely accepted as being a demonstrable entity, does not in fact exist. The depression in the lining of the anal canal noted by these authors in the living subject is not produced by the main insertion of the modified longitudinal muscle coat of the bowel wall at this site, but results from contraction of the subcutaneous portion of the external sphincter.

(2) As the level of this depression marks precisely the interval between the internal sphincter and the subcutaneous sphincter, the term anal intermuscular depression could properly be used to describe it. Moreover, such a term would be in harmony with the main point made by Hilton (1863) when he first directed attention to this 'lineal' interval.

(3) There is a well-marked layer of involuntary muscle and connective tissue in the submucosa of the anal canal from the level of the anal sinuses to the intersphincteric interval. While commonly ignored it would appear that this tissue layer must play an important part in determining the clinical appearances presented by haemorrhoids. In this latter connexion the surgical conception of submucous and perianal spaces was considered.

**Interference microscopy of cells in tissue culture.** By M. ABERCROMBIE, E. J. AMBROSE, D. M. EASTY and J. E. M. HEAYSMAN. *University College and Chester Beatty Research Institute, London*

The interference microscope shows ranges of cell thickness and of protein concentration as differences in colour. Time-lapse colour filming of tissue cultures, using this microscope, has proved to be a useful technique for precise observation of cell behaviour and interactions. Such a film was shown. Amongst the phenomena which can be observed are the undulating motion of the membranous edge of a fibroblast, associated with pinocytosis; the substantial arrest of this motion where two cells are in contact; the tendency of fibroblasts when moving on a plane surface to remain as a single layer except during mitosis; and the great activity at the surface of a rounded tumour cell.

**Investigation of a connective tissue constituent in scabs.** By D. W. JAMES. *University College, London*

Hydroxyproline is an amino-acid found in quantity principally in connective tissue fibres, but considerable amounts have been reported in the scab formed over healing cutaneous wounds in rats (James, D. W., *J. Path. Bact.* **69**, 1955). It has been suggested that this may indicate the presence of a collagen precursor in the scab, since no fibres are demonstrable histologically. The present work concerns the further investigation of scab hydroxyproline.

It appeared probable that hydroxyproline-containing material is drawn into the scab from the underlying repair region, the scab losing fluid from its free surface and acting as a wick. A series of cutaneous wounds in rats, inflicted with a standard technique (Abercrombie, M., Flint, M. H. & James, D. W., *J. Emb. exp. Morph.* **2**, 1954) was dressed with absorbent cellulose. After 3 days the dressing was removed, hydrolysed with 6 N-HCl, and shown to contain hydroxyproline. In a further series of wounds similar absorbent dressings were covered with impermeable polythene sheet, and at 3 days no hydroxyproline was found in hydrolysates of the dressings. These results suggest that hydroxyproline-containing material may be drawn into the scab from the subjacent repair region as the scab dries.

At present no proof can be offered of the hypothesis that the scab contains collagen precursors rather than breakdown products from mature fibres injured at the time of wounding but Harkness, R. D. *et al.* (1954) have described the isolation of procollagens from rabbit skin, and it was thought of interest to determine whether scab material contained hydroxyproline fractions having comparable solubilities to described procollagens. Scabs from standard wound have been pooled to give weights of material in the region of 100 mg., and hydroxyproline has been shown to be extracted in part from the material by both citrate (pH 3.8) and phosphate (pH 9.0) buffers. The greater part of the scab hydroxyproline is, however, insoluble in these buffers, but can be brought into solution by autoclaving at 30 lb. pressure for 6 hr. in aqueous solution.

The significance of these results was discussed.

**The structure of normal human epidermis and the distribution of melanocytes.** By G. SZABÓ. *London Hospital Medical School*

Thiersch grafts were split with trypsin (Medwar, 1941) and subsequently the pure epidermal sheets treated with 'dopa'. As stated in a preliminary report (Szabó, 1954), there is a wide range of variation of the number of melanocytes between corresponding areas on



different individuals, but, as a rule, there are 2000–4000 melanocytes per mm.<sup>2</sup> of skin surface in the face, forehead and ears, whereas on other areas the number varies between 500 and 2000. Their number shows a gradual decline during postnatal development. Naturally hyperpigmented areas, for example, the areola of the nipple, contain melanocytes in no higher numbers than the surrounding skin: thus qualitative changes of melanocytes rather than their increase in number is responsible for hyperpigmentation.

Split epidermis is also suitable for studying the pattern of epidermal ridges and the distribution of epidermal appendages. It was found that the number of appendages per mm.<sup>2</sup> of skin declined during postnatal development at a higher rate in areas which are not exposed than on exposed areas. The small number of melanocytes in unexposed areas of skin may therefore be due to the different rates of 'stretching' of skin regions rather than to the fact that they are not exposed to sunshine: the melanocytes thus being placed farther apart on the thigh than on the face.

#### **Pineal homografts in rabbits.** By R. L. HOLMES. *University of Leeds*

Homografts of adult and young (1 month) pineal tissue have been made into the anterior chamber of the eye in a small series of adult rabbits.

Examination at periods of up to 3 months after operation has shown either that: (a) the graft failed to 'take', became necrotic and disappeared; or (b) the graft became white and opaque, and histologically showed poor vascularization, with cellular infiltration, and gross changes in pineal cells; or (c) the graft became pink, and histologically showed good vascularization with persistence of pineal cells and retention of nuclear characteristics.

In some grafts, increase in size of pineal cell nuclei was seen, along with variations in the staining properties of the cell bodies. The cytology of these and other persisting pineal cells has been compared with that of cells in the normal tissue, and the relationship between connective tissue elements and pineal parenchymal cells in the grafts described.

#### **Changes in regional lymph nodes induced by homo- and heterografts of cartilage in rabbits.** By M. B. L. CRAIGMYLE. *University College, Cardiff*

It has been established that the presence of an antigenic substance in a tissue will cause enlargement of, and cytological changes within, the regional lymph node draining the tissue. These effects are the accompaniment of antibody formation by the node (Fagraeus, 1948). It has also been established that skin homografts elicit an antibody response in the host (Medawar, 1944, 1945) and produce similar enlargement and cytological changes in the regional node (Scothorne, 1954). Cartilage homografts and heterografts are known to survive transplantation for long periods, and one reason advanced has been that they do not elicit an antibody response.

Whole and chopped homografts of cartilage were implanted subcutaneously at the base of one ear in nineteen rabbits and the regional lymph nodes excised during a period of from 3 to 21 days. No significant enlargement of the nodes on the operated sides occurred during this time. Similar tests were carried out on four rabbits using fresh guinea-pig cartilage and in four rabbits using preserved bovine cartilage. These grafts produced a slight enlargement of the regional nodes on the operated sides after a few days. A second set of homografts and heterografts performed on ten rabbits produced considerable enlargement of the nodes on the operated sides within a few days following the second implantation.

Every node was studied histologically after staining with the methyl green/pyronin method. The cytological changes were described. The significance of these findings was discussed in relation to the use of cartilage grafts in plastic surgery.

**Uptake of radioactive calcium by fracture callus.** By J. J. PRITCHARD and F. GIRGIS. *Queen's University, Belfast*

In a previous communication by one of us, it was shown that, while radioactive calcium was readily taken up by fracture callus, as demonstrated autoradiographically, if administered in the diet, or by injection, very little was mobilized from the general skeleton and incorporated in the callus when the skeleton had previously been rendered radioactive by administration of  $^{45}\text{Ca}$  in the diet *before* fracture. In the present experiments rats, whose bones had previously been made radioactive, were given a low calcium diet during the course of fracture repair and compared with similarly treated rats on a normal diet. Autoradiographs of the fracture site showed that considerable amounts of  $^{45}\text{Ca}$  were incorporated in the callus by mobilization from the skeleton in the rats on the low calcium diet. The callus was more radioactive still when a large dose of parathyroid hormone was administered to rats on a low calcium diet. The results suggest that the calcium needed for repair is derived principally from the diet, under normal conditions, but that endogenous sources are readily available when the diet is deficient. Parathormone therapy, with the object of ensuring calcification of fracture callus, while possibly effective, would appear to be unnecessary in the presence of a normal diet.

**An analysis of the growth rate of the liver in the foetal sheep.**

By D. A. T. DICK. *University of Glasgow*

It is well known that the relative weight of the liver in the foetal sheep declines from about 12 % of the body weight at the end of the first month of development to between 3 and 4 % at full term. Determinations of the total number of hepatic cells in fifty-one foetal livers at different stages from 36 days to full term show that this relative decrease in the amount of liver substance is real and not a mere reduction in the haemopoietic tissue in the liver. The number of hepatic cells present per 100 g. of body weight is about  $16 \times 10^8$  at 36 days and decreases to  $6 \times 10^8$  at full term. During a similar period the relative growth rate of the body by weight also declines from 22 % per day to 2 % per day. There is a linear correlation between these two quantities over the period observed which suggests a causal relationship between them. For instance, the number of liver cells present per 100 g. of body weight at any stage may be accounted for by a constant number, approximately  $5 \times 10^8$ , presumably required to maintain normal metabolism plus a variable number, proportional to the bodily growth rate at the time and presumably required to maintain growth. This theory implies that the metabolic activity of the individual's hepatic cell remains the same throughout the period investigated. Attempts have been made to test this assumption by cytological and cytochemical methods.

**Morphological status of cranial sutures.** By F. GIRGIS and J. J. PRITCHARD. *Queen's University, Belfast*

Up to the present it has been impossible to decide whether the sites of the cranial sutures are determined by the meeting of the growing edges of the individual bones, or whether their position is determined independently and prior to the meeting of the bones. Previous attempts to solve this problem experimentally have involved removal and subsequent regeneration of portions of the cranial vault *after* the position of the sutures has been determined. In the present study damage has been produced by cautery to the skull vault of rats *in utero*, *before* the establishment of the sutures. Subsequently, asymmetrical suture patterns have developed which are unrelated either to the configuration of the venous sinuses, or the topographical asymmetry of the damaged brain, and it is evident that compensatory overgrowth of one bone into the normal territory of a neighbouring bone, has occurred. These results suggest that the position of the sutures is determined by the meeting of the individual bones, and not independently.

**Cone-shaped epiphyses of the proximal phalanges of the toes.** By P. VENNING  
(introduced by J. Z. YOUNG). *University College, London*

The epiphyses of the proximal phalanges of the 2nd-5th toes sometimes develop in the form of a cone with the apex directed distally into the proximal end of the diaphysis. Radiographs of the feet of samples of children show that these conic epiphyses occur most commonly on the 3rd toe and least commonly on the 5th toe. They are commoner among females than males. They fuse with the diaphysis earlier than the non-conic epiphysis. They are correlated with the absence of an epiphysis of the middle phalanx of the same toe. The order of frequency of conic epiphyses among the toes corresponds to the usually reported order of ossification of the epiphyses of the proximal phalanges.

A possible relation between the development of cone-shaped epiphyses, pseudo-epiphyses and the congenital absence of epiphyses is discussed.

**Electromyographic observations on some of the muscles of mastication.** By B. E. GREENFIELD and B. D. WYKE (introduced by G. CAUSEY). *Royal College of Surgeons, London*

The results of electromyographic studies of the masseter and temporal muscles in normal subjects were shown. Monopolar skin electrodes were employed, the compound spike potentials being recorded by means of a four-channel ink-writing electroencephalograph. The activity of these muscles was shown during habitual biting in the centric position, and also during eccentric, protrusive and retrusive biting. The records from different parts of the muscles during these movements were demonstrated. The activity in similar muscles on the two sides, and in different muscles on the same side was compared by simultaneous recording.

The muscle activity in a patient with abnormal closure of the jaws was demonstrated.

**Regeneration of the articular surfaces of the mandibular joint in rabbits.**  
By R. SPRINZ. *University of Sheffield*

The excision of the mandibular condyle and the zygomatic process of the squamosal bone was followed by their regeneration within 4 weeks in adult rabbits. Operations were performed unilaterally and bilaterally. In outline the new joint surfaces were similar to, though less regular than, the originals. The site of the new articulation was on the same plane as the original. The appearance of the regenerated mandibular condyles was similar to that of the condyles following meniscectomy. The articular meniscus became fused to the operated side of the joint, thereby reducing the two joint cavities to one.

The post-operative condition was studied with reference to the well-being of the animal, and the effect of the operation on the dentition.

**The immobility of the lower edge of the internal oblique and conjoined tendon.**  
By F. S. A. DORAN. *Surgeon, Mid-Worcestershire Hospitals*

The description of the action of the internal oblique and transversus muscles given in the standard text-books pays no particular attention to their lower borders. Keith (1923), however, stated that they descended, like a 'shutter' on to the inguinal ligament to protect the inguinal canal. The standard operations for the cure of hernia are based on this concept of a 'shutter'. A consideration of the attachments of those fibres which arise from the inguinal ligament and which form the conjoined tendon suggest that the 'arch' they make over the spermatic cord is virtually rigid. This suggestion is confirmed by observation during surgical operations on the groin and by the insertion of silver markers, which later were X-rayed with the muscles relaxed and then contracted.



**A Bronze Age burial near Stonehaven, Kincardineshire.**By J. McKENZIE. *University of Aberdeen*

An exceptionally fine example of a Bronze Age short stone cist was found recently in a big, natural tumulus on a farm 3 miles south-west of Stonehaven. Lying at a depth of 5 ft. below the surface, the undisturbed contents of the cist were beautifully displayed upon dislodgement of one of the side walls, and consisted of the skeletal remains of a young woman about 20–25 years of age and of a newborn child, three intact food vessels or beakers typical of that age, and several flints and worked stones.

Photographs of the cist and its contents before they were removed were shown, and the skeletal remains described.

**The morphology of the respiratory portion of the lateral wall of the nasal cavity.** By C. C. D. SHUTE. *University of Cambridge*

The snout region has been studied in serial sections of late embryonic and post-natal stages of various primitive placentals.

The nasal cavity of mammals possesses a lateral recess whose aperture is bounded by the crista semicircularis above and the maxilloturbinal below and which is divided by the lateral stem of the ethmoturbinal complex into an upper olfactory compartment containing ectoturbinals and a lower maxillary sinus which is non-olfactory and buds off glandular tissue.

In some, but not all, mammals the maxillary hiatus is bounded inferiorly by an uncinat process, which varies in its mode of formation. In *Elephantulus* the uncinat process is continuous anteriorly with the crista semicircularis, but in *Cynocephalus* (= *Galeopithecus*) it is derived from the base of the lateral ethmoturbinal stem. The evidence is against the commonly held view that the agger nasi and uncinat process of man represent a nasoturbinal.

Between the uncinat process and the maxilloturbinal the glandular tissue derived from the maxillary sinus commonly becomes confluent with the lateral nasal gland. The latter is not formed in man. In *Sorex* it is very prolific, and invades the crista semicircularis and the ethmoturbinal stem, recalling the inclusion of the homologous gland within the nasal concha of lizards.

The primitive function of the maxillary sinus appears to be to act as a receptacle for glandular secretions, and that of the maxillo- and nasoturbinals to overhang the nasolacrimal duct and the duct of the lateral nasal gland respectively.

**Further observations on the colon primum of the sheep.**By R. N. SMITH. *University of Bristol*

The second part of the colon primum ('ascending colon') of the sheep is usually coiled on itself in a spiral fixed to the common mesentery. The various regular forms of this spiral found in a survey of over a thousand specimens have been recorded elsewhere (Smith, *J. Anat., Lond.*, **89**, 1955). However, about 20 % of the specimens examined showed some irregularity in the coiling and were not analysed in that communication.

Of these irregular patterns approximately 25 % showed a U-, S- or Z-shaped deviation occurring only in the last coil. The remainder showed a variety of forms, some occurring several times, many of which could be classified into apparent sequences.

These various irregularities were shown and very briefly discussed.

**Abnormal origin of the basilar artery from the cervical part of the internal carotid and its embryological significance.** By D. B. MOFFAT and E. D. MORRIS.  
*University College, Cardiff*

In a 67-year-old dissecting-room subject the basilar artery arose from the left internal carotid artery at the level of the second cervical vertebra and entered the skull through the anterior condylar canal. This anomalous basilar artery was joined in the posterior cranial fossa by the two extremely small vertebral arteries.

The left vertebral artery arose from the arch of the aorta and entered the foramen transversarium of the fifth cervical vertebra; the right had a double origin from the right subclavian and ascending cervical arteries. The brachial plexus was post fixed.

It was suggested that the anomalous artery represents the persistence of the hypoglossal artery.

The three cases previously described in the literature support this view.

**The development of the cremaster muscle.** By H. BUTLER and K. M. BACKHOUSE.  
*St Bartholomew's Hospital Medical College, London*

It has long been known (John Hunter, 1762) that the human cremaster muscle is inverted while the testis remains intra-abdominal, i.e. its fasciculi ascend cranialwards towards the caudal pole of the testis. Examination of embryos and foetuses of several placental mammalian species (man, pig, sheep, rat, rabbit, seal, mole and bat) shows that the cremaster muscle first develops in the inverted position. In the sheep and pig the cremaster muscle attains its adult (everted) position before the testis traverses the inguinal canal. In the opossum (*Didelphys aurita*) and the phalanger (*Trichosurus vulpecula*) the cremaster muscle is never inverted and displays the adult (everted) arrangement from its earliest appearance. This is associated with a large and precocious processus vaginalis. The significance of these facts was discussed in relation to the mechanism of testicular descent.

**Induction effects of early fracture callus grafted under kidney capsule in the rabbit.** By J. B. BRIDGES and J. J. PRITCHARD. *Queen's University, Belfast*

From time to time suggestions have been made that induction phenomena play an important role in bone repair. In order to test this hypothesis, fracture callus has been grafted under the kidney capsule in young rabbits. Fractures were produced manually in the tibia, and 9–12 days later portions of the centre of the callus mass at the fracture site were removed and grafted, some still living, some after immersion in absolute alcohol for 24 hr., under the kidney capsule of the same animal. At the time of grafting the callus was at the fibrocartilaginous stage of differentiation. Living autografts rapidly became organized into a lens-shaped ossicle of cancellous bone. Dead autografts, however, underwent gradual resorption, but in their vicinity an ovoid mass of cartilage and endochondral bone developed from the surrounding fibrous tissue. The significance of induction by fibrocartilaginous callus in the course of bone repair, was discussed.

**A specimen with unusual congenital anomalies of the cardiovascular system.**  
By H. MIDDLETON and B. TOWERS. *University of Cambridge*

A premature (35-week) infant with multiple congenital abnormalities lived for 30 min. after birth. The heart and great vessels show the following anomalies: the right atrium receives (a) the hepatic vein (which has normal communication with the umbilical vein), (b) just above this opening, a common channel for the upper and lower right and the lower left pulmonary veins, and (c) a rather small right superior vena cava. The left atrium

receives (*a*) a common channel draining a persistent left superior vena cava and upper left pulmonary vein, and (*b*) the inferior vena cava. There is free inter-atrial communication via a foramen secundum, there being no evidence of a septum secundum. Both atria open into an enlarged right ventricle, the opening being bounded by a small right and a large left valve cusp. There is complete atresia of the pulmonary trunk, and the right ventricle opens, through deficiencies in the inter-ventricular septum, into a small left ventricle and large aortic vestibule. The aorta is right-sided, and the lungs are supplied *via* a patent ductus arteriosus which connects the aorta to the right pulmonary artery. Various features suggest partial transposition of viscera. The embryological significance of the findings was discussed.



## INDEX TO VOLUME 89

- Abd-el-Malek, S. Part played by tongue in mastication and deglutition, 250
- Adrenal gland, vascularization of, in rhesus monkey. By R. G. Harrison and C. W. Asling, 106
- Adreno-cortical histogenesis in rat. By J. D. Lever, 293
- Aitken, R. N. C. Histochemical study of seminal vesicle of sheep, 430
- Anastomoses, arterio-venous, in head and ears of calf. By A. M. Goodall, 100
- Ansa spiralis coli of sheep. By R. N. Smith, 246
- Arterial supply of human prostate and seminal vesicles. By E. J. Clegg, 209
- Asling, C. W. *See* Harrison, R. G., joint authors, 106
- Autoradiographs of incorporation of methionine in mouse tissues. By A. Glucksmann, A. Howard and S. R. Pelc, 13
- Barnett, C. H. Factors influencing angulation of neck of mammalian talus, 225
- Barnicot, N. A. and Hardy, R. H. Position of hallux in West Africans, 355
- Barr, M. L. *See* Lindsay, H. A., joint authors, 47
- Biceps femoris, insertion of. By R. S. Sneath, 550
- Biggers, J. D. and Claringbold, P. J. Mitotic activity in vaginal epithelium of mouse following local oestrogenic stimulation, 124
- Billingham, R. E. and Medawar, P. B. Growth in healing of wounds in mammalian skin, 114
- Bone, influence of cortisone on. By H. A. Sissons and G. J. Hadfield, 69
- Book Reviews:
- Analysis of Development. Ed. by B. H. Willier, P. A. Weiss and V. Hamburger. Reviewed by A. Hughes, 555
- Anatomy of the Bronchial Tree. By R. C. Brock. Reviewed by R. J. Harrison, 268
- Anatomy of the Bronchovascular System. By G. L. Birnbaum. Reviewed by R. J. Harrison, 268
- An Introduction to Cell and Tissue Culture. Ed. W. F. Scherer. Reviewed by A. Hughes, 554
- Atlas postmortale Angiogramme. By J. Schoenmackers and H. Vieten. Reviewed by P. Daniel, 556
- Biochemistry of Semen. By T. Mann. Reviewed by D. V. Davies, 422
- Cytoarchitecture of Human Brain Stem. By J. Olszewski and D. Baxter. Reviewed by J. D. Boyd, 424
- Die Entwicklung und Morphologie des chondrocraniums von *Myotis*. By H. Frick. Reviewed by J. D. Boyd, 132
- Prefrontal Leucotomy and Related Operations. By A. Meyer and E. Beck. Reviewed by F. Goldby, 422
- Primates. Part 2. Haplorhini: Tarsioidea. By W. C. Osman Hill. Reviewed by W. E. Le G. Clark, 554
- Vertebrate Dissection. By W. F. Walker, Jun. Reviewed by H. L. H. Green, 423
- Boyd, J. D. Book review. Cytoarchitecture of Human Brain Stem. By J. Olszewski and D. Baxter, 424
- Book review. Die Entwicklung und Morphologie des chondrocraniums von *Myotis*. By H. Frick, 132
- Brain of humpback whale, surface features of. By A. S. Breathnach, 343
- Breathnach, A. S. Endocranial casts of cetaceans, 532
- Surface features of brain of humpback whale, 343
- Broncho-pulmonary segments in sheep. By W. C. D. Hare, 387
- Calf, arterio-venous anastomoses in head and ears of. By A. M. Goodall, 100
- Campbell, E. J. M. Scalene and sternomastoid muscles in breathing, 378
- Carman, J. B. Carotid labyrinth in *Hyla* and *Leiopelma*, 503
- Carotid labyrinth in *Hyla* and *Leiopelma*. By J. B. Carman, 503
- Cartilage, epiphyseal, effects of excision, in rabbit. By P. A. Ring, 79
- Cartilage, epiphyseal, excision and reimplantation of, in rabbit. By P. A. Ring, 231
- Cat, hypothalamic neurosecretion in. By J. C. Sloper, 301
- Cat, intracellular lipid in kidney of. By M. C. Lobban, 92
- Cat, organization of visual cortex in. By D. A. Sholl, 33
- Cat, placodal relations of neural crest in. By G. Halley, 133
- Cell degeneration during normal ontogenesis of rabbit brain. By B. Källén, 153
- Cetaceans, endocranial casts of. By A. S. Breathnach, 532
- Claringbold, P. J. *See* Biggers, J. D., joint authors, 124
- Clark, W. E. Le G. Book review. Primates. Part 2. Haplorhini: Tarsioidea. By W. C. Osman Hill, 554
- Clegg, E. J. Arterial supply of human prostate and seminal vesicles, 209
- C.N.S., mammalian, perivascular spaces of. By D. H. M. Woollam and J. W. Millen, 193
- Cocker, R. and Hatton, J. M. Innervation of human dentine, 189
- Cortex, neo-, cell types in. By N. L. Mitra, 467
- Cortex, visual, organization of, in cat. By D. A. Sholl, 33
- Cortisone, influence of, on microglia. By E. J. Field, 201
- Cortisone, influence of, on structure and growth of bone. By H. A. Sissons and G. J. Hadfield, 69

- Daniel, P. Book review. Atlas postmortale Angiogramme. By J. Schoenmackers and H. Vieten, 556
- Davies, D. V. Book review. Biochemistry of Semen. By T. Mann, 422
- Davis, P. R. Thoraco-lumbar mortice joint, 370
- Dental pulp, blood supply of odontoblast layer of. By W. Warwick James, 547
- Dentine, human, innervation of. By R. Cocker and J. M. Hatton, 189
- Dog, enumeration of glomeruli in kidney of. By R. V. Sellwood and E. B. Verney, 63
- Dog, hypothalamic neurosecretion in. By J. C. Sloper, 301
- Duran-Jorda, F. Renal ducts of Bellini, 464
- Ear, inner, selective staining of. By G. B. Wislocki and A. J. Ladman, 3
- Electromyographic study of scalene and sternomastoid muscles in breathing. By E. J. M. Campbell, 378
- Endocranial casts of cetaceans. By A. S. Breathnach, 532
- Epiphysis, distal ulnar, of rabbit, ossification and growth of. By P. A. Ring, 457
- Esterase activity in skin of mammals. By W. Montagna and V. R. Formisano, 425
- Field, E. J. Development of microglia and influence of cortisone, 201
- Formisano, V. R. *See* Montagna, W., joint authors, 425
- Glomeruli, enumeration of, in kidney of dog. By R. V. Sellwood and E. B. Verney, 63
- Glucksmann, A., Howard, A. and Pele, S. R. Incorporation of methionine in mouse tissues as indicated by autoradiographs. I. Testis, epididymis and seminal vesicle, 13
- Goldby, F. Book review. Prefrontal Leucotomy. By A. Meyer and E. Beck, 422
- Goodall, A. M. Arterio-venous anastomoses in skin of head and ears of calf, 100
- Green, H. L. H. H. Book review. Vertebrate Dissecting. By W. F. Walker, Jun., 423
- Guillery, R. W. Quantitative study of mamillary bodies and their connexions, 19
- Hadfield, G. J. *See* Sissons, H. A., joint authors, 69
- Halley, G. Placodal relations of neural crest in domestic cat, 133
- Hallux, position of, in West Africans. By N. A. Barnicot and R. H. Hardy, 355
- Hardy, R. H. *See* Barnicot, N. A., joint authors, 355
- Hare, W. C. D. Broncho-pulmonary segments in sheep, 387
- Harrison, R. G. and Asling, C. W. Vascularization of adrenal gland in rhesus monkey, 106
- Harrison, R. J. Book review. Anatomy of the Bronchial Tree. By R. C. Brock, 268
- Book review. Anatomy of the Broncho-vascular system. By G. L. Birnbaum, 268
- Hatton, J. M. *See* Cocker, R., joint authors, 189
- Howard, A. *See* Glucksmann, A., joint authors, 13
- Hughes, A. Book review. Introduction to Cell and Tissue Culture. Ed. W. F. Scherer, 554
- Book review. Analysis of Development. Ed. by B. H. Willier, P. A. Weiss and V. Hamburger, 555
- Hypothalamic neurosecretion in dog and cat. By J. C. Sloper, 301
- In Memoriam:  
Keith, Sir Arthur, 403  
Whillis, James, 419  
Wood Jones, Frederic, 255
- Jacoby, F. and Martin, B. F. Alkaline phosphatase in obstructive jaundice, 440
- James, W. Warwick. Blood capillary system of odontoblast layer of dental pulp, 547
- Johnson, F. R. and McMinn, R. M. H. Implantation grafts of bladder mucosa, 450
- Joint, carpo-metacarpal of thumb. By J. R. Napier, 362
- Joint, thoraco-lumbar mortice. By P. R. Davis, 370
- Källén, B. Cell degeneration during normal ontogenesis of rabbit brain, 153
- Keen, E. N. Postnatal development of human cardiac ventricles, 484
- Keith, Sir Arthur, *In memoriam* notice by J. C. Brash, and A. J. E. Cave, 403
- Krohn, P. L. Autografts and homografts of vaginal tissue in rabbits, 269
- Ladman, A. J. *See* Wislocki, G. B., joint authors, 3
- Lever, J. D. Adreno-cortical histogenesis in rat, 293
- Lindsay, H. A. and Barr, M. L. Behaviour of nuclear structures during depletion and restoration of Nissl material, 47
- Lipid, intracellular, in kidney of cat. By M. C. Lobban, 92
- Lobban, M. C. Intracellular lipid in kidney of cat, 92
- Lymph nodes, cellular changes in, following skin homografting. By R. J. Scothorne and I. A. McGregor, 283
- McGregor, I. A. *See* Scothorne, R. J., joint authors, 283
- McKenzie, J. Morphology of sternomastoid and trapezius muscles, 526
- McMinn, R. M. H. *See* Johnson, F. R., joint authors, 450
- Mamillary bodies, quantitative study. By R. W. Guillery, 19
- Martin, B. F. *See* Jacoby, F., joint authors, 440
- Medawar, P. B. *See* Billingham, R. E., joint authors, 114
- Methionine, incorporation of, in mouse tissues. By A. Glucksmann, A. Howard and S. R. Pele, 13
- Microglia, development of, and influence of cortisone. By E. J. Field, 201
- Millen, J. W. *See* Woollam, D. H. M., joint authors, 193
- Mitotic activity in vaginal epithelium of mouse. By J. D. Biggers and P. J. Claringbold, 124

- Mitra, N. L. Cell types in neo-cortex, 467
- Monkey, rhesus, vascularization of adrenal gland in. By R. G. Harrison and C. W. Asling, 106
- Montagna, W. and Formisano, V. R. Esterase activity in skin of mammals, 425
- Mouse, mitotic activity in vaginal epithelium of. By J. D. Biggers and P. J. Claringbold, 124
- Muscles, scalene and sternomastoid, in breathing. By E. J. M. Campbell, 378
- Muscles, sternomastoid and trapezius, morphology of. By J. McKenzie, 526
- Napier, J. R. Carpo-metacarpal joint of thumb, 362
- Neural crest, placodal relations of, in cat. By G. Halley, 133
- Neurosecretion, hypothalamic, in dog and cat. By J. C. Sloper, 301
- Nuclear structures, behaviour of during depletion and restoration of Nissl material. By H. A. Lindsay and M. L. Barr, 47
- Odontoblast layer of dental pulp, blood supply of. By W. Warwick James, 547
- Pallie, W. *See* Weddell, G., joint authors, 162, 175
- Palmer, E. *See* Weddell, G., joint authors, 162
- Pele, S. R. *See* Glucksmann, A., joint authors, 13
- Perivascular spaces of mammalian C.N.S. and their relation to perineuronal and subarachnoid spaces. By D. H. M. Woollam and J. W. Millen, 193
- Phosphatase, alkaline, in guinea-pigs with obstructive jaundice. By F. Jacoby and B. F. Martin, 440
- Placodal relations of neural crest in cat. By G. Halley, 133
- Prostate, human, arterial supply of. By E. J. Clegg, 209
- Rabbits, autografts and homografts of vaginal tissue in. By P. L. Krohn, 269
- Rabbit, cell degeneration in brain of, during development. By B. Källén, 153
- Rabbit, cellular changes following skin homografting in. By R. J. Scothorne and I. A. McGregor, 283
- Rabbit, effects of excision of epiphyseal cartilage in. By P. A. Ring, 79
- Rat, adreno-cortical histogenesis in. By J. D. Lever, 293
- Rat, alkaline phosphatase in developing teeth of. By N. B. B. Symons, 238
- Renal ducts of Bellini. By F. Duran-Jorda, 464
- Ring, P. A. Effects of partial or complete excision of epiphyseal cartilage of rabbit, 79
- Excision and reimplantation of epiphyseal cartilage of rabbit, 231
- Ossification and growth of distal ulnar epiphysis of rabbit, 457
- Scalene and sternomastoid muscles in breathing. By E. J. M. Campbell, 378
- Scothorne, R. J. and McGregor, I. A. Cellular changes in lymph nodes and spleen following skin homografting in rabbit, 283
- Sellwood, R. V. and Verney, E. B. Enumeration of glomeruli in kidney of dog, 63
- Seminal vesicles, arterial supply of. By E. J. Clegg, 209
- Seminal vesicle, of sheep, histochemistry. By R. N. C. Aitken, 430
- Sheep, ansa spiralis coli of. By R. N. Smith, 246
- Sheep, broncho-pulmonary segments in. By W. C. D. Hare, 387
- Sholl, D. A. Organization of visual cortex in cat, 33
- Silver, P. H. S. Valves of Houston in human embryo and foetus, 217
- Sissons, H. A. and Hadfield, G. J. Influence of cortisone on structure and growth of bone, 69
- Skin homografting, cellular changes following. By R. J. Scothorne and I. A. McGregor, 283
- Skin, innervation of. I. Origin, course and number of sensory nerves supplying rabbit ear. By G. Weddell, W. Pallie and E. Palmer, 162
- Skin, innervation of. II. Number, size and distribution of hairs and hair follicles in rabbit. By G. Weddell and W. Pallie, 175
- Skin, innervation of. III. Patterned arrangement of spinal sensory nerves to rabbit ear. By G. Weddell, D. A. Taylor and C. M. Williams, 317
- Skin, mammalian, contracture and intussusceptive growth in healing of extensive wounds in. By R. E. Billingham and P. B. Medawar, 114
- Skin, of mammals, esterase activity in. By W. Montagna and V. R. Formisano, 425
- Sloper, J. C. Hypothalamic neurosecretion in dog and cat, 301
- Smith, R. N. Arrangement of ansa spiralis coli of sheep, 246
- Sneath, R. S. Insertion of biceps femoris, 550
- Spleen, cellular changes in, following skin homografting. By R. J. Scothorne and I. A. McGregor, 283
- Symons, N. B. B. Alkaline phosphatase activity in developing teeth of rat, 238
- Talus, mammalian, factors influencing neck of. By C. H. Barnett, 225
- Taylor, D. A. *See* Weddell, G., joint authors, 317
- Teeth, of rat, developing, alkaline phosphatase in. By N. B. B. Symons, 238
- Thoraco-lumbar mortice joint. By P. R. Davis, 370
- Thumb, carpo-metacarpal joint of. By J. R. Napier, 362
- Tongue, part played by, in mastication and deglutition. By S. Abd-el-Malek, 250
- Vaginal tissue, in rabbits, autografts and homografts of. By P. L. Krohn, 269
- Valves of Houston in human embryo and foetus. By P. H. S. Silver, 217
- Ventricles, human cardiac, postnatal development of. By E. N. Keen, 484



- Verney, E. B. *See* Sellwood, R. V., joint authors, 63
- Weddell, G., Pallie, W. and Palmer, E. Studies on innervation of skin. I. Origin, course and number of sensory nerves supplying the rabbit ear, 162
- Weddell, G. and Pallie, W. Studies on innervation of skin. II. Number, size and distribution of hairs and hair follicles in rabbit ear, 175
- Weddell, G., Taylor, D. A. and Williams, C. M. Studies on innervation of skin. III. Patterned arrangement of spinal sensory nerves to rabbit ear, 317
- Whale, humpback, brain of. By A. S. Brethnach, 343
- Whillis, James, *In memoriam* notice by T. B. Johnston, 419
- Williams, C. M. *See* Weddell, G., joint authors, 317
- Wislocki, G. B. and Ladman, A. J. Selective and histochemical staining of otolithic membranes, cupulae and tectorial membrane of inner ear, 3
- Wood Jones, Frederic, *In memoriam* notice by W. E. Le Gros Clark, 255
- Woollam, D. H. M. and Millen, J. W. Perivascular spaces of mammalian C.N.S., 193

## SUPPLEMENTARY INDEX OF PROCEEDINGS

- Abercrombie, M., Ambrose, E. J., Easty, D. M. and Heayman, J. E. M. Interference microscopy of cells in tissue culture, 575
- Adrenal autografts, cortical zonation and survival of chromaffin cells in. By R. F. Coupland, 561
- Aitken, J. T. Larger anterior horn cells in cat's lumbar cord, 571
- Ambrose, E. J. *See* Abercrombie, M., joint authors, 575
- Anal canal, anatomy of. By E. W. Walls, 574
- Anterior horn cells, larger, in cat's lumbar cord. By J. T. Aitken, 571
- Arteries, 'polypoid formations' in. By D. B. Moffat, 560
- Aumonier, F. J. Lower birth canal in rabbit, 566
- Backhouse, K. M. *See* Butler, H., joint authors, 580
- Bacsich, P. and Wyburn, G. M. Subcutaneous homografts of xiphoid cartilage, 560
- Barlow, T. E. Variations in blood supply of jejunum, 560
- Basilar artery, abnormal origin of. By D. B. Moffat and E. D. Morris, 580
- Bellairs, A. d'A. Interorbital septum in chick embryos, 568
- Biggs, P. M. and King, A. S. Respiration through humerus of *Gallus*, 567
- Birbeck, M. S. C. *See* Howe, A., joint authors, 574
- Birth canal, lower, minute structure of, in rabbit. By F. J. Aumonier, 566
- Bladder mucosa transplants and heterotopic bone formation. By F. R. Johnson and R. M. H. McMinn, 559
- Bouton terminaux* of cervical region of cat spinal cord. By G. W. Pearce and P. Glees, 563
- Bowden, Ruth E. M. Relations of recurrent laryngeal nerve in neck, 564
- Bridges, J. B. and Pritchard, J. J. Induction effects of early fracture callus, 580
- Broncho-pulmonary segment concept in *Artiodactyla* and *Carnivora*. By D. Brown, 561
- Brown, D. Broncho-pulmonary segment concept, 561
- Burial, bronze age. By J. McKenzie, 579
- Butler, H. and Backhouse, K. M. Development of cremaster muscle, 580
- Callus, early fracture, induction effects of. By J. B. Bridges and J. J. Pritchard, 580
- Callus, fracture, uptake of radioactive calcium by. By J. J. Pritchard and F. Girgis, 577
- Cardiovascular system, congenital anomalies of. By H. Middleton and B. Towers, 580
- Cauna, N. Nerve endings in Meissner's corpuscle, 568
- Cauna, N. Structure and development of Meissner's corpuscle, 562
- Causey, G. and Hofman, H. Relation of Schwann cell to nerve fibre, 562
- Cells, interstitial, of Cajal, in guinea-pig ileum. By J. McKenzie, H. W. Kosterlitz and J. A. Robinson, 573
- Cerebral cortex, of rat, cell territories in. By H. Maturana, 572
- Colon primum of sheep. By R. N. Smith, 579
- Connective tissue constituent in scabs. By D. W. James, 575
- Cortical neurons, surface area of. By D. A. Sholl, 571
- Cortisone, effects of on neonatal rat. By E. J. Field, 559
- Coupland, R. E. Cortical zonation and survival of chromaffin cells in adrenal autografts, 561
- Cowan, W. M. and Powell, T. P. S. Projection of midline and intralaminar nuclei of thalamus, 574
- Cowan, W. M. *See* Powell, T. P. S., joint authors, 563
- Craigmyle, M. B. L. Lymph node changes induced by grafts, 576
- Cranial sutures, morphological status of. By F. Girgis and J. J. Pritchard, 577
- Cremaster muscle, development of. By H. Butler and K. M. Backhouse, 580
- Cysts of developmental origin in oral cavity of human foetuses. By J. H. Scott, 561
- Davis, P. R. Movements of human lower thoracic and lumbar vertebrae, 565
- Dawson, I. M. and Wyburn, G. M. Nissl's substance, cytoplasmic filaments and nuclear membrane of spinal ganglion cells, 573
- Dental tubules, fibres in. By M. A. MacConaill, 569
- Dick, D. A. T. Growth rate of foetal sheep liver, 577
- Doran, F. S. A. Immobility of lower edge of internal oblique and conjoined tendon, 578
- Easty, D. M. *See* Abercrombie, M., joint authors, 575
- Electromyographic records and their interpretation. By J. Joseph, 559
- Enarthrodial plates, mechanics and function of. By M. A. MacConaill, 566
- Epidermis, human, and distribution of melanocytes. By G. Szabó, 575
- Epiphyses, cone-shaped. By P. Venning, 578
- Epithelium, transitional, regenerating. By R. M. H. McMinn and F. J. Johnson, 570
- Evans, D. H. L. and Hamlyn, L. H. Fibre degeneration methods in nervous system, 572
- Evans, E. Gwynne and Tulley, W. J. Orofacial muscles and dental alignment, 557
- Facial muscles, afferent nerve supply of. By Z. Y. Mahran, 564
- Field, E. J. Effects of cortisone on neonatal rat, 559



- Genital tract, maturation of, in female kitten. By P. P. Scott, 565
- Girgis, F. and Pritchard, J. J. Morphological status of cranial sutures, 577
- Girgis, F. *See* Pritchard, J. J., joint authors, 577
- Glees, P. *See* Pearce, G. W., joint authors, 563
- Glycogen in developing pharyngeal derivatives of sheep. By R. J. Scothorne, 557
- Greenfield, B. E. and Wyke, B. D. Electromyography of muscles of mastication, 578
- Hamlyn, L. H. *See* Evans, D. H. L., joint authors, 572
- Hand, human, prehensile movements of. By J. R. Napier, 564
- Harrison, T. J. Role of sacro-iliac joint in growth of pelvis, 558
- Heayman, J. E. M. *See* Abercrombie, M., joint authors, 575
- Hippocampus-fornix system, fibre degeneration following lesions of. By T. P. S. Powell and W. M. Cowan, 563
- Hofman, H. *See* Causey, G., joint authors, 562
- Holmes, R. L. Pineal homografts in rabbits, 576
- Homografts, subcutaneous, of xiphoid cartilage. By P. Bacsich and G. M. Wyburn, 560
- Howe, A., Richardson, K. C. and Birbeck, M. S. C. Mitochondria in mammary gland of guinea-pig, 574
- Interference microscopy of cells in tissue culture. By M. Abercrombie *et al.*, 575
- Internal oblique and conjoined tendon, immobility of lower edge of. By F. S. A. Doran, 578
- Interorbital septum in chick embryos. By A. d'A. Bellairs, 568
- James, D. W. Connective tissue constituent in scabs, 575
- Jejunum, variations in blood supply of. By T. E. Barlow, 560
- Johnson, F. R. *See* McMinn, R. M. H., joint authors, 570
- Johnson, F. R. and McMinn, R. M. H. Bladder mucosa transplants and heterotopic bone formation, 559
- Joseph, J. Electromyographic records and their interpretation, 559
- King, A. S. *See* Biggs, P. M., joint authors, 567
- Kosterlitz, H. W. *See* McKenzie, J., joint authors, 573
- Lee, I. N. Sympodia, 570
- Liver, growth rate of, in foetal sheep. By D. A. T. Dick, 577
- Lloyd-Jacob, M. A. and Scott, P. P. Vaginal smear technique in cat, 565
- Lymph node changes induced by grafts. By M. B. L. Craigmyle, 576
- MacConaill, M. A. Fibres in dentinal tubules, 569
- MacConaill, M. A. Mechanics and function of enarthrodial plates, 566
- McKenzie, J. Anatomy and embryology of Treacher Collins syndrome, 558
- Bronze age burial, 579
- McKenzie, J., Kosterlitz, H. W. and J. A. Robinson. Function of interstitial cells of Cajal, 573
- McMinn, R. M. H. and Johnson, F. R. Regenerating transitional epithelium, 570
- McMinn, R. M. H. *See* Johnson, F. R., joint authors, 559
- Mahran, Z. Y. Afferent nerve supply of facial muscles, 564
- Mandible, regeneration of articular surfaces of, in rabbits, By R. Sprinz, 578
- Maturana, H. Cell territories in cerebral cortex of rat, 572
- Meissner's corpuscle, nerve endings in. By N. Cauna, 568
- Meissner's corpuscle, structure and development of. By N. Cauna, 562
- Middleton, H. and Towers, B. Congenital anomalies of cardiovascular system, 580
- Mitchell, G. A. *See* Warwick, R., joint authors, 562
- Mitochondria, in mammary gland of guinea-pig. By A. Howe, K. C. Richardson and M. S. C. Birbeck, 574
- Moffat, D. B. 'Polypoid formations' in arteries, 560
- Moffat, D. B. and Morris, E. D. Abnormal origin of basilar artery, 580
- Morris, E. D. *See* Moffat, D. B., joint authors, 580
- Morton, W. R. M. and Scott, J. H. Skeletal remains from burial mound, 567
- Mystacial vibrissae, of rat and cat, blood supply of. By M. G. Scott, 566
- Napier, J. R. Prehensile movements of human hand, 564
- Nasal cavity, morphology of respiratory portion of lateral wall of. By C. C. D. Shute, 579
- Nervous system, study of fibre degeneration methods in. By D. H. L. Evans and L. H. Hamlyn, 572
- Optic chiasma of cephalopods. By J. P. Stanier and J. Z. Young, 571
- Orofacial muscles and dental alignment. By E. Gwynne Evans and W. J. Tulley, 557
- Osman Hill, W. C. Peritoneal specialization in *Saimiri*, 569
- Pearce, G. W. and Glees, P. *Boutons terminaux* of cervical region of cat spinal cord, 563
- Periotic duct. By C. C. D. Shute, 569
- Peritoneal specialization in *Saimiri*. By W. C. Osman Hill, 569
- Phrenic nucleus of rhesus monkey. By R. Warwick and G. A. Mitchell, 562
- Pineal homografts in rabbits. By R. L. Holmes, 576
- Powell, T. P. S. and Cowan, W. M. Fibre degeneration following lesions of hippocampus-fornix system, 563
- Powell, T. P. S. *See* Cowan W. M., joint authors, 574
- Pritchard, J. J. and Girgis, F. Uptake of calcium by fracture callus, 577



- Pritchard, J. J. *See* Bridges, J. B., joint authors 580  
 — *See* Girgis, F., joint authors, 577
- Recto-vesical and recto-vaginal pouches in human embryo. By P. H. S. Silver, 568  
 Recurrent laryngeal nerve, relations of in neck. By Ruth E. M. Bowden, 564  
 Respiration through humerus of *Gallus*. By P. M. Biggs and A. S. King, 567  
 Richardson, K. C. *See* Howe, A., joint authors, 574  
 Robinson, Judith A. *See* McKenzie, J., joint authors, 573
- Sacro-iliac joint, role of, in growth of pelvis. By T. J. Harrison, 558  
 Schwann cell, relation of, to nerve fibre. By G. Causey and H. Hofman, 562  
 Scothorne, R. J. Glycogen in developing pharyngeal derivatives of sheep, 557  
 Scott, J. H. Cysts of developmental origin in oral cavity of human fetuses, 561  
 — *See* Morton, W. R. M., joint authors, 567  
 Scott, M. G. Blood supply of mystacial vibrissae of rat and cat, 566  
 Scott, Patricia P. Maturation of genital tract of female kitten, 565  
 — *See* Lloyd-Jacob, M. A., joint authors, 565  
 Sertoli cells *in vivo* and *in vitro*. By G. A. Thomas, 557  
 Sholl, D. A. Surface area of cortical neurons, 571  
 Shute, C. C. D. Morphology of lateral nasal wall, 579  
 — Secondary tympanic membrane and periotic duct, 569  
 Silver, P. H. S. Recto-vesical and recto-vaginal pouches in human embryo and fetus, 568  
 Skeletal remains from burial mound. By W. R. M. Morton and J. H. Scott, 567  
 Smith, R. N. Colon primum of sheep, 579  
 Spinal ganglion cells, Nissl's substance, cytoplasmic filaments and nuclear membrane of. By I. M. Dawson and G. M. Wyburn, 573  
 Sprinz, R. Regeneration of mandibular articular surfaces, 578  
 Stanier, J. P. and Young, J. Z. Optic chiasma of cephalopods and significance of crossed tracts, 571  
 Symptodia. By I. N. Lee, 570  
 Szabó, G. Human epidermis and distribution of melanocytes, 575
- Thalamus, of rabbit, projection of nuclei of. By W. M. Cowan and T. P. S. Powell, 574  
 Thomas, G. A. Sertoli cells *in vivo* and *in vitro*, 557  
 Towers, B. *See* Middleton H., joint authors, 580  
 Treacher Collins syndrome. By J. McKenzie, 558  
 Tulley, W. J. *See* Evans, E. Gwynne, joint authors, 557  
 Tympanic membrane, secondary. By C. C. D. Shute, 569
- Vaginal smear technique, in cat, value of. By M. A. Lloyd-Jacob and P. P. Scott, 565  
 Venning, P. Cone-shaped epiphyses of proximal toe phalanges, 578  
 Vertebrae, human, movements of lower thoracic and lumbar. By P. R. Davis, 565
- Walls, E. W. Anatomy of anal canal, 574  
 Warwick, R. and Mitchell, G. A. Phrenic nucleus of rhesus monkey, 562  
 Wyburn, G. M. *See* Baesich, P., joint authors, 560  
 — *See* Dawson, I. M., joint authors, 573  
 Wyke, B. D. *See* Greenfield, B. E., joint authors, 578
- Young, J. Z. *See* Stanier, J. P., joint authors, 571